The behaviour and ecology of Alfred mantas (*Manta alfredi*) in the Maldives

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Abstract

At the commencement of this study all data on *Manta* rays were combined under the single species *Manta birostris*, and there was little information available on their ecology. Manta rays were frequently reported throughout the Maldives, and tourist manta-watching is an economically important activity in this region although no previous study of manta numbers or movements had been made.

An individual identification method was developed based on the unique ventral marking pattern of each animal in order to record the identity and frequency of visit by mantas to the numerous cleaning and feeding areas. Information on sex, estimated size, and the markings pattern were collated in an Access database along with date/time and location of each animal sighting. Mantas were also observed and recorded photographically and on video being cleaned by cleaner fish and during feeding activity so that typical behaviours could be investigated.

Analysis of the ID records and sightings data of over 1900 individually identified mantas has shown that the smaller reef-associated *Manta alfredi* is the predominant species in the Maldives. The majority of mantas (~74%) have been sighted only once, but this is probably due to a relatively low survey rate, with the remainder re-sighted between one and 34 times. Individual mantas which were re-sighted appeared confined to an atoll, visiting a number of cleaning stations around the atoll and migrating between east and west sides so as to be predominantly on the leeside to the prevailing monsoon. Only ~1% of mantas were reported in more than one different atoll with 270 km being the greatest distance between sites where an individual manta was reported, a range not previously recorded.

A population study estimated populations of around 537 mantas in small atolls and 811 in large atolls. The population was ~65% female, and females were significantly larger than males. Reproductive periodicity appears low with only 26% of likely mature females ever being sighted pregnant over a 5 year period. Females which were reported pregnant produced offspring less frequently than every two years, on average. This low reproductive rate might put this small population at risk if local fishing practices changed.

The behaviour of mantas at cleaning stations was investigated as well as the identity and abundance of cleaner fish species. Feeding strategies are described and appear to show that feeding is a co-operative behaviour in mantas. These preliminary behavioural studies provide early insights into the ecology of this species.

Acknowledgements

I extend my sincere thanks to my main supervisor Prof. A. J. Edwards for providing considerable guidance throughout this project. I would also like to thank my second supervisor Dr R. Bevan for direction in the behavioural chapters in this thesis.

The majority of research was carried out whilst I was working on MVs Sea Queen, Sea Spirit and Gaaviya. I wish to express my thanks to Rob Bryning and Sam Harwood of Maldives Scuba Tours for allowing me to conduct my research whilst working on the boats. The crews of all three boats worked very hard to support the diving operation in order to conduct the research and I would especially like to thank the captains: Hassan, Mohammed Rasheed, Anwar and Mohammed.

There have been many who have assisted me with my data collection who must be acknowledged. Firstly there was Erica Norman who was there back in 2001 when I first started to question just how many mantas we were seeing every week and with whom I collaborated with on the original PADI Manta Speciality. Video and photographs used for manta identification have been contributed by (hundreds of) tourist divers who were diving with us and I thank them for their interest and involvement. Of special mention are the contributors M. Shafraz Naeem, Lisa Allison and Guy Stevens who helped plug some holes when I was away from the Maldives, and I also thank them for their shared enthusiasm and camaraderie. There were many others who helped but I would especially like to mention Nick and Demelza Masters for their help during “Manta Weeks” on Sea Spirit, Dave and Lisa Allison, Tracy and David Allen and finally Chas Anderson for his suggestion that I *do* something with the all the accumulated data.

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Finally I would like to express my thanks to my husband Matt Kitchen for his understanding and involvement in this project. Without his technical and emotional support this project would not have come to fruition.

Statement of the Contribution of Others

Chapter 1 includes information from a paper published in *Tourism in Marine Environments* (a copy is in Appendix A) of which I was a co-author and provided significant data on diving activities, sites visited, etc. I was closely involved in the discussion on the valuation of manta diving in the Maldives with the main author Dr R. C. Anderson. In Chapter 2 the manta size/weight graph was initially created by Prof. A. J. Edwards. The Access database introduced in Chapter 3 was designed by Prof. Edwards but all data entry and maintenance was my own. The main ID method in Chapter 4 was published in *Marine Biology Research* and two reviewers made minor recommendations for changes which were also incorporated into the final version of this Chapter. The Chapter has been amended several times since publication of the paper and the results and discussion expanded. A copy of the paper is in Appendix A. Chapter 6 has been published as a paper in *Environmental Biology of Fishes* (I was lead author) and three anonymous reviewers and co-authors Dr C. Ari and Prof. Edwards improved upon earlier versions. The Chapter is based on the paper but has been amended. Dr Ari provided the data for observations on 16 to 18th August 2009 at Hanifaru used in the Chapter; all other data are my own. The paper was written by me with feedback and comments from co-authors (a copy is in Appendix A). Prof. Edwards conducted the Rayleigh analyses used in chapter 7 to investigate manta activity at cleaning stations with tide and one set of the data used in Chapter 10 used to investigate the mating seasons hypothesis, I performed the other. Guy Stevens and I pooled data on mantas sighted pregnant between 2001 and 2007 used in chapter 10 (the quantity of data provided by each of us was roughly equal), but all analyses were my own.

Sightings data collected since 2000 used in chapters 4, 5, and 7 to 10 were obtained by me or my husband Matt Kitchen who operated the video camera. Additional video and photographs used for manta identification were contributed by (hundreds of) tourist divers and dive guides who were diving with us and assisted with specific areas of data collection under my direction. A small amount (<5%) of data were contributed by other guides and divers who were not diving with us, the main contributors were M. Shafraz Naeem, Lisa Allison and Guy Stevens who volunteered the photographs and videos with full knowledge of the use of their material in this body of work.

Table of Contents

List of Figures

List of Tables

Chapter 1. Introduction

1.1 Origin and objectives of the project

The project arose from the personal interest of the author in the mantas observed weekly as part of a tourist diving itinerary. Observations developed into an organised study as the database of photographically identified individual mantas grew, and experience in observing mantas increased. By the time the project was formalised and proposed in 2005 the database of identified mantas already exceeded 600 individuals. The project was managed and funded by the author with volunteer divers assisting on surveys during their scuba diving holidays in the Maldives.

The principal objectives were: (1) to determine the range of sizes, size at sexual maturity, ratio of males to females and to describe the typical colour variant in the observed population; (2) investigate migration behaviour as mantas were typically only seen on the lee-side of the atolls; (3) estimate the population of mantas in studied atolls (North Male, Ari and Baa atolls) and for the entire Maldives; (4) investigate the commonly observed behaviours of mantas whilst they were being cleaned and during feeding and (5) investigate social interactions and reproductive behaviour. The expectation was that with a better understanding of manta behaviours the times and places to observe mantas could be predicted more accurately, and the impacts of observers on the natural activities of mantas would be minimised. The initial objectives list evolved from observations. Once it was determined that it was not the same 20 mantas seen during all the observation periods at a particular cleaning station, I wanted to identify how many individual mantas were using the cleaning station during an observation period, during a day, and estimate the size of the local population. Thus, the objectives were constantly reviewed and some objectives reconsidered and amended as other issues came to the fore and required investigation. Some objectives were disregarded when it became apparent that investigation was beyond the scope of this project. A perceived strength of this study was that the research period was relatively unlimited. A broad range of investigations were conducted during 9 years of study with initial results stimulating new research directions.

1.2 Background

At the commencement of this study in 2001 there were little information on the ecology of species of *Manta* (Bancroft 1829) available. The genus was considered to include a

single species: the giant manta *Manta birostris* (Walbaum 1792) (Bigelow and Schroeder 1953; Eschmeyer et al. 1983; Compagno 1999b). This confused information gathering on the species most commonly seen in the Maldives, more recently considered to be Alfred manta *Manta alfredi* (Krefft 1868) (Marshall et al. 2009). As a result, much of the information in the literature e.g. maximum size, size at maturity, migration patterns etc. appeared at variance with my observations of *M. alfredi* in the Maldives. At commencement of this study the only information available to interested amateurs were species profiles in fish identification books and accounts of interactions with mantas at cleaning and feeding areas written for divers in specialist diving magazines and tourist guide books. Despite a great number of casual observations of mantas by divers, no systematic population or migration studies had been undertaken in Maldives other than small-scale manta tagging (Anderson 1996). There was no information about likely population size, lifespan or reproductive cycles in the literature. Information on size in available texts seemed incorrect compared to the author's observations due to texts dwelling on maximum rather than typical size, which referred to the larger species (*M. birostris*) rather than that common in the Maldives (*M. alfredi*) (Last and Stevens 1994; Henneman 2001; Passarelli and Piercy 2005).

Once the species were separated (Marshall et al. 2009) the literature could be reviewed and the species identified in each reference. The meagre literature on the ecology of mantas is reviewed in Chapter 2. Until recently there was little useful published data on manta ecology or behaviour. Concurrent research was being undertaken on the ecology of *M. alfredi* in Mozambique, Hawaii (both Maui and Big Island), French Polynesia and Western Australia and by another researcher in the Maldives. Additionally, a study of *M. birostris* was being carried out in the eastern Pacific by two groups of researchers and another, in the Gulf of Mexico. The first Devil Ray symposium was hosted by the American Elasmobranch Society at the Joint Meeting of Ichthyologists and Herpetologists, in Montreal, Canada in July 2008. The increased activity in manta research was likely to result in a significant body of published data becoming available by the latter stages of this study.

When this project commenced in 2001, the primary objective was to identify individual mantas visiting cleaning stations, so as to be able to recognise individuals and determine how many individuals visited a specific cleaning station during each survey. An identification method was developed over a period of around three months which is

described in Chapter 4. The research project was formalised in 2005 and the original paper records transferred to a specially created Access database for ease of analysis. Use of the database is described in Chapters 3 and 4. The results of analysis of individual manta's marking patterns supported new evidence that there were two or more species (Marshall et al. 2009) with *M. alfredi* being confirmed as the commonly seen species in the Maldives, with occasional reports of *M. birostris*. The results, along with the identification methodology, were reported in Kitchen-Wheeler (2010).

Information from the database included records of over 1900 different mantas which provided valuable evidence on population composition, observed sizes, range and movements and aspects of social and reproductive behaviours. This was by far the largest dataset available for any location in the world. The project is presented as a series of studies with each study being covered in a Chapter. The topic of Chapters 3 and 5 to 10 are introduced in the next section.

1.3 Economic importance of mantas in the Maldives and rationales for study

The Republic of the Maldives is a small island nation in the tropical Indian Ocean, southwest of India (Figure 1.1), comprising 26 coral atolls and approximately 1200 islands. The geography of the Maldives is covered in more detail in Chapter 3.

The Maldivian economy is based almost entirely on fisheries and tourism with the latter bringing in 60% of foreign exchange receipts and 90% of tax revenue. Tourism is heavily influenced by the country's geography, lending to the development of exclusive island resorts of which there were 98 operating at the end of 2008 plus 140 registered 'safari boat' vessels (Ministry of Tourism Arts and Culture 2009). Scuba diving and snorkelling have been a key component of Maldives tourism since its inception in 1972. The importance of diving has declined in recent years with the development of more up-market resorts. The Ministry of Tourism and Civil Aviation (2007) reported than an estimated 15% of tourists still visited primarily for diving and that other tourists made some dives and went snorkelling. It was recognised that the success of Maldivian tourism depended on the marine environment and there was awareness of the importance of environmental issues and the need for sustainability within the industry. The Maldives' *National Biodiversity Strategy and Action Plan* (Ministry of Home Affairs Housing and Environment 2002) emphasised the importance of biodiversity conservation and called for economic evaluation of ecologically and socially important components of biodiversity.

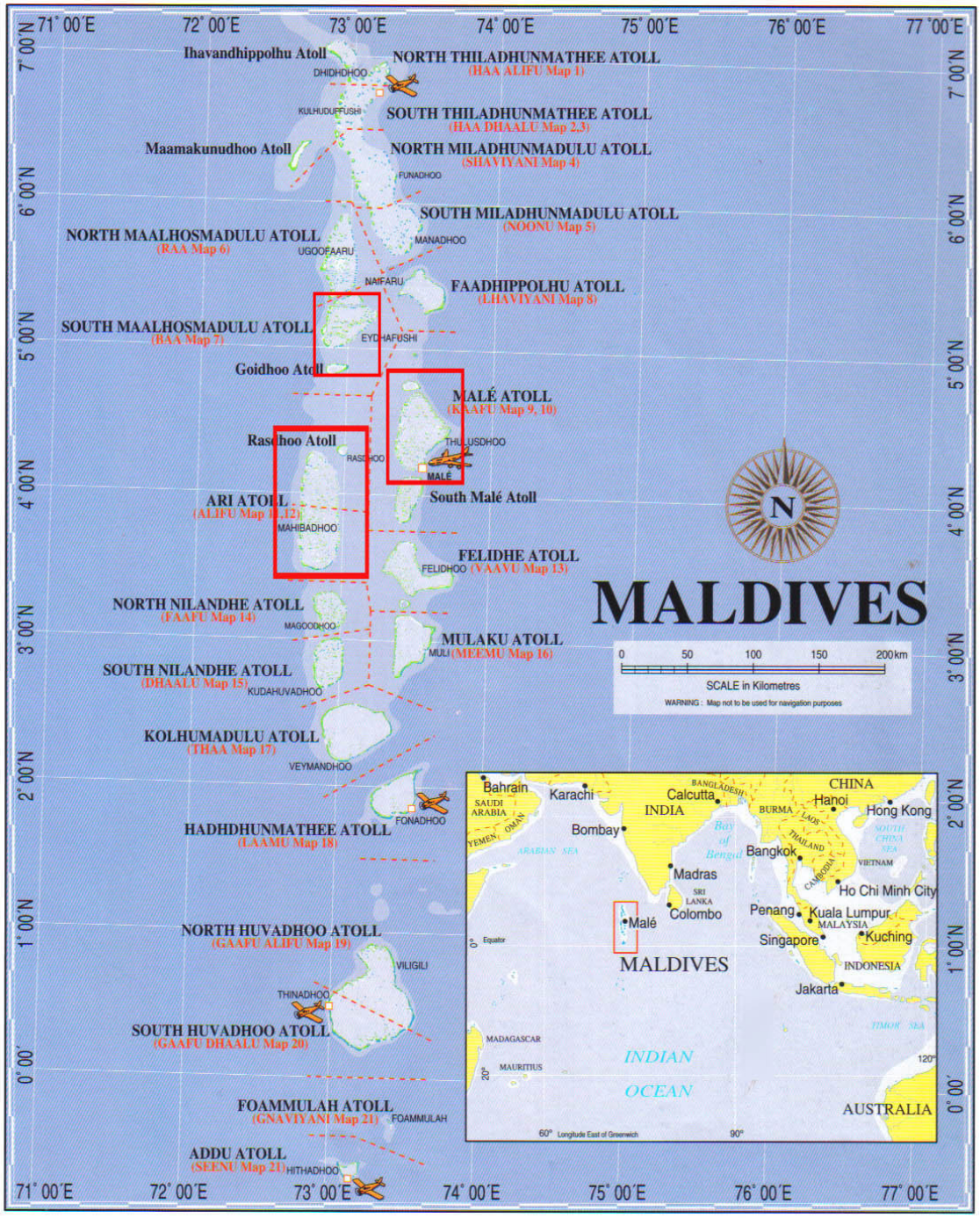

Figure 1.1 The atolls of the Maldives are located to the south-west of India (small inset map). Note the chain splits into two parallel chains between Noonu (North Miladhunmadulhu) and Thaa (Kolhumadulu) atolls. The three main study areas of North Male, Ari and Baa are highlighted. From Atlas of the Maldives (Godfrey, 2004)

The importance of mantas in the culture of the Maldives is evident in the number of geographical locations named after the genus. There are at least 12 islands and at least as many channels with the word *Madi* in the title (Godfrey 2004). *Madi* is the *Dhivehi* (Maldivian language) word for ray and usually refers to manta ray when another species

word is not added. The name *Madivaru* is common for a place (a channel or island) where mantas are frequently seen. *Madivaru* literally translates to “manta point” and the latter is used to denote scuba diving or snorkelling-with-manta sites within the tourism industry in the Maldives.

The unique geography of the Maldives combined with the monsoonal weather pattern appear to influence many marine species with the main issues affecting *M. alfredi* discussed in Chapter 3 and a study of the movement of mantas, likely caused by the monsoon seasons, reported in Chapter 5.

There has been no commercial fishery for mantas in living history and mantas were only rarely caught by Maldivian fishermen whose target catch are the commercially important skipjack and yellowfin tuna species (*Katsuwonus pelamis* (Linnaeus 1758) and *Thunnus albacares* (Bonaterre 1788)) for both local consumption and export. Fishing of mantas does occur in the Lakshadweep (Koya 1998), mainland southern India (Kunhipalu and Boopendranath 1981) and Sri Lanka continues. In the decade since 2000, rapid growth in the harvesting of manta meat and gill-rakers had been reported. The meat was used as a shark-fin substitute and the gill-rakers in modern and traditional Chinese medicine (Shen et al. 2001; Pope 2009). With little or no knowledge of migration and reproductive cycles and life spans of manta populations the continued fishing of mantas in neighbouring countries could pose a threat to Maldives populations. A study of the reproductive behaviours of *M. alfredi* is reported in Chapter 10.

Knowledge on worldwide manta populations and movements is important due to the tourism income from snorkelling and diving with mantas. Tourist diving with mantas is also economically important in several places throughout the world where mantas are regularly encountered e.g. Hawaii, Yap, East Africa, Polynesia, Indonesia, and the Caribbean (Anderson et al. 2010). In the Maldives diving with sharks and rays was stated as the primary goal for most diving visitors (Anderson and Waheed 2001), thus diving with mantas is a very important activity as it contributes significantly to the income from tourism. In addition to the lack of a commercial (or artisanal) manta fishery in the Maldives, shark and ray fishing was banned in tourist atolls (Male, Ari, Baa, Lhaviyani) in 1995 to protect the elasmobranchs for the enjoyment of sport divers (Anderson 1998). The ban was extended to all Maldivian territorial waters in 2009 (Omidi 2009). It was expected that demand for diving with sharks and rays would grow in line with the growth in tourist numbers (Figure 1.2) so it was important to evaluate

the impact of diving on the behaviour of local populations of animals. A study of atoll populations of mantas in the central atolls is presented in Chapter 6. Both the population study and Access database were useful in providing a baseline estimate of manta numbers and identification of the individual mantas which might be used to measure the future impacts from tourism or environmental changes. Understanding the mantas' daytime feeding and cleaning behaviours was important as these are the main activities which bring mantas into contact with man. The presence of divers will have affected the behaviour of mantas as the mantas had to accommodate the presence of large numbers of humans (divers, snorkelers) at their feeding and cleaning areas or to abandon these areas. However, the changes were not all necessarily negative. New cleaning and feeding behaviours have evolved from the presence of divers and the development of local and resort islands. Chapters 7 and 8 report observations of manta cleaning and feeding activity in the Maldives and include examples of behaviours which have been caused by, or developed from, economic development within the country. Chapter 9 is a study of manta social behaviours and reports preliminary observations of group social behaviour and the most frequently encountered intraspecific interactions. Knowledge of these may be useful for predicting behaviour in response to the presence of humans, and together with the knowledge on the commonly encountered cleaning and feeding behaviours may be used to minimise human impact on natural manta behaviour.

Data from this study contributed to an investigation of the economic value of manta tourism in the Maldives. The report by Anderson et al. (2010) identified 91 manta sites throughout the Maldives where an estimated 143,000 dives and 14,000 snorkels were made annually during 2006-8. These were valued at about US$8.1 million per year in direct revenue derived from US$45 or US$70 per scuba dive (depending on the site) and US$20 per snorkeler (Table 1.1). The indirect value of manta tourism was likely to be substantially greater when the costs of equipment hire, food and beverages, accommodation and travel to or around the Maldives to visit mantas were taken into account. For the direct contribution valuation, care was taken not to overestimate by excluding data from sites dived for a small part of the season and using a fee of only US$45 for closely located resorts and US$70 for more distant resorts. A single excursion at an upmarket resort could cost US$750 or more. With growth in tourist numbers and increasing costs, this value would be expected to grow significantly in the near future. This income provided salaries for local divemasters, boat skippers, boat builders, dive centre staff etc. The diving industry is a major employer of local workers

and knowledge of best sites whilst securing healthy manta populations for the future will help secure the livelihoods of these individuals.

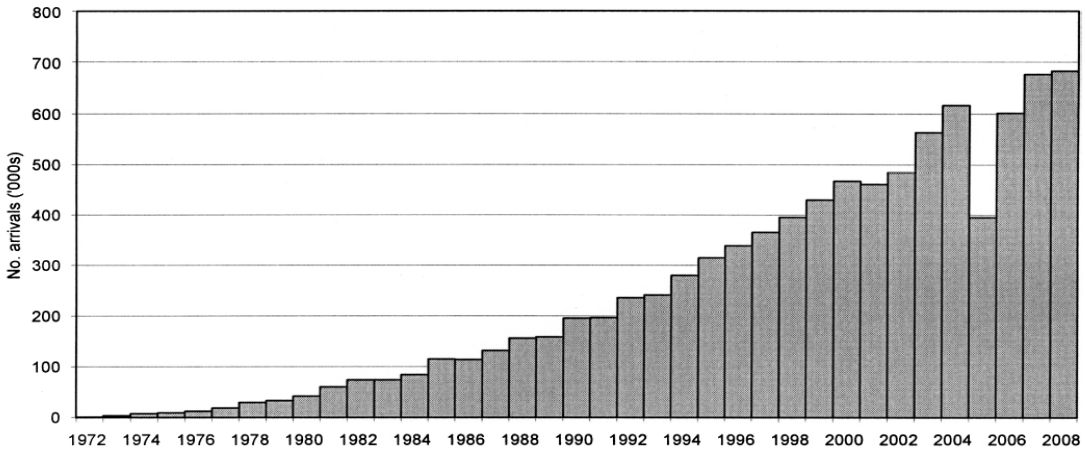

Figure 1.2 Annual tourist arrivals in the Maldives, 1972-2008 compiled from annual reports of Ministry of Tourism (Anderson et al. 2010).

Administrative atoll	No. sites	No. boat trips made each year	No. dives made each year	Estimated value
Haa Alifu	5	210	1,700	$119,000
Raa	5	132	1,188	$58,320
Baa	12	2,411	19,230	$1,087,550
Lhaviyani	3	442	5,310	$269,550
North Male	18	3,423	36,231	$1,798,700
South Male	9	998	11,068	$602,900
North Ari	13	1,183	14,796	$880,560
South Ari	15	3,770	44,840	$2,494,200
Felidhoo	6	685	7,045	$409,275
Mulaku	2	90	810	$40,500
North Nilandhe	1	32	256	$14,080
Addu	2	60	480	$26,400
Total	91	13,436	142,954	$7,801,035

Table 1.1 Estimated annual extent and value ($US) of diving-with-mantas, per atoll. Estimates were based on US$45 or US70$ per scuba dive and US$20 per snorkel (Anderson et al. 2010).

Manta diving was most valuable in North Male, South Ari and Baa atolls, which combined, accounted for nearly 70% of revenue (Table 1.1). These atolls were the most developed and had the highest concentrations of resorts (64% of total resorts) and visitors. Future growth is likely to come from developing atolls like Huvadhoo and Laamu (not included in the economic study as nil economically active resorts) plus increased diving intensity in atolls such as Haa Alifu which had only one safari boat and

two resorts for inclusion in the economic study. The major manta ray diving sites identified in the report were all included in this study.

Tourism is very important to the Maldives economy and its success is heavily dependent on the good condition of the marine environment. The government has stated its intention to evaluate biodiversity. This study includes important insights into the ecology and behaviour of *Manta alfredi,* an economically important species, compiled by a scientist who works in the tourism industry and has an interest in both the conservation and the economic potential of this animal.

Chapter 2. The genus *Manta*: taxonomy, morphology and review of literature

2.1 Abstract

The amalgamation of two or more species of *Manta* in *Manta birostris* has resulted in all observations in the past 50 years being ascribed to *M. birostris* despite differences in morphology, size, colour and migratory behaviour. Literature was reviewed and information pertinent to species were separated where a species could be identified. When a photograph was not available the only indicator of species was location or size, but it was generally possible to predict species based on descriptions of physical characteristics, geographical location, behaviour etc. and this literature review supports recent research that there are two or more *Manta* species.

Two species, *Manta alfredi* and *Manta birostris*, can be defined by the physical characteristics of maximum size, dorsal and ventral marking patterns, tooth rows in only lower, or both jaws (respectively) and the presence of a postdorsal protuberance (*M. birostris* only). Dorsal and ventral markings patterns are particularly useful for species identification in the field and were more fully investigated in this study. The size: weight relationships of both species appear similar. The ranges of the two species are not fully known although there appears to be some sympatry. A third species, *Manta* sp. cf. *birostris*, is the subject of current research.

Manta alfredi is reported to reach at least 36 years of age, to have a gestation period of 12-13 months in the wild, be capable of consecutive pregnancies and be sexually mature at a size of 4.1 m disc width (DW) for females and 3.7 m DW for males. Three different movement patterns have been reported in populations of mantas. *Manta alfredi* were reported as resident to an area, swimming distances of around 20 km between feeding and cleaning areas, but being seen year-round at these locations. A few individuals of *M. alfredi* were also reported to migrate between sites several of hundred kilometres apart driven by changes in seasons. Individual *M. birostris* were reported travelling between sites hundreds of kilometres apart but re-sightings of individuals in the same location were rare.

2.2 Introduction

Despite their large size and frequent sightings in near-shore circum-tropical habitats there is very little published about rays of the genus *Manta* (Bancroft 1829). The genus was considered monospecific with all mantas being designated *Manta birostris* (Walbaum 1792) since the mid-20th century, despite the same researchers also expressing some doubt due to regional variations in size and markings patterns in particular (Bigelow and Schroeder 1953; Eschmeyer et al. 1983; Compagno 1999b). Difficulty with reconciling my field observations with information in reference monographs for *Manta birostris* was the catalyst for this research, since much of the information available was contradicted by field data. For example, my observations of maximum size and size at sexual maturity were much smaller than those stated in main references (Last and Stevens 1994; Henneman 2001; Passarelli and Piercy 2005; McEachran 2006). Descriptions of behaviours were also contradictory e.g. in Henneman (2001) *Manta birostris* was described as placid enough to allow divers "to hitch a ride" and "should better not be touched". I began collecting data for this project in 2001 to investigate the size range, migration activity and behaviour of mantas observed in the Maldives. Since commencement of this research, the taxonomy of *Manta* species has been revised (Marshall et al. 2009) and it is now apparent that many of these contradictions are due to two or more species of manta being present.

Earlier manta scientific literature was reviewed in light of current views on *Manta* species (Marshall et al. 2009). The information in this Chapter presents a review of most available information on *Manta* species collected from the literature, peer reviewed websites and personal communications.

2.3 Taxonomy

Manta rays are in the family Myliobatidae (eagle and devil rays) and subfamily Mobulinae (Homma et al. 1999). The Mobulinae (devil rays) include two genera – *Manta,* and *Mobula* Rafinesque 1810 with 9 species (Notarbartolo-Di-Sciara 1987). The terminal position of the mouth of the *Manta*, extending across the front of its head instead of on its lower surface, distinguishes it from *Mobula.* Until recently the accepted view as exemplified by Eschmeyer (2008) was that there was only one worldwide species of *Manta* with the thirteen nominal species described all being synonyms, and thus all research in recent years has been ascribed to *Manta birostris* (Walbaum 1792).

The giant manta, *Manta birostris*, was first described by Walbaum (1792) as *Raja birostris*, with no locality given. Some authors, e.g. Whitley (1936), regarded Walbaum's treatment as non-binominal, and thus dated the first description to Donndorff (1798). However, Eschmeyer (2008) noted that Walbaum's style suggested that the second word "birostris" was not italicized through an oversight and binominal nomenclature was intended. The currently accepted name is thus *Manta birostris* (Walbaum 1792). Morphological studies by Marshall et al. (2009) indicated that at least two species, which can be separated on the basis of tooth row counts, teeth in both or the lower jaw only, presence/absence of a spine and postdorsal protuberance, dorsal and ventral markings, behaviour and size, may be present. This hypothesis appears to be supported by DNA evidence (Kashiwagi et al. 2009). The smaller species (< 5 m disc width - DW) is *Manta alfredi* (Krefft 1868) whereas the larger species (which grows to more than 5 m DW) appears to correspond to *Manta birostris*.

The Alfred manta, *Manta alfredi*, was first described from Watson's Bay, Sydney, Australia by Krefft (1868) as *Deratoptera alfredi.* Whitley (1936) noted the difference between *Manta birostris* and *Manta alfredi* (under the synonyms *Deratoptera alfredi*, *Ceratoptera alfredi* and *Manta alfredi*), with the latter having a different shape of pectoral fin and different dentition. He wrote "there being over 200 rows of teeth in lower jaw" in *M. alfredi*. However, he probably intended to write files (the size of the line of teeth forming a row); of which there are 142-182 teeth in each file, in 6-8 rows in the lower jaw only, according to Marshall et al. (2009). Both species have teeth but the dentition in *M. alfredi* is more pronounced: *M. birostris* has enlarged dermal denticles (less pronounced teeth) in both jaws. Whitley renamed the genus *Daemomanta* in 1932 and refers to the species as *Daemomanta alfredi*. A third putative species, referred to as *Manta sp. cf. birostris* by Marshall et al. (2009) may be distinct from *M. birostris.* They suggest that the name *Manta giorna* be resurrected for it, based on *Cephalopterus giorna* Lesueur 1824. This putative species appears to be distinguished from *M. birostris* by its distinctive dorsal and ventral markings and to be endemic to the Caribbean and north-west Atlantic. Interestingly, Bigelow and Schroeder (1953), when discussing the known species of *Manta*, commented: "The Mantas from all parts of the world resemble one another so closely in general appearance that all of the supposed species that have been named from the western Pacific-Indian Ocean region have been united recently with the Atlantic *Manta birostris* under that name. But *Manta hamiltoni* of the Pacific Coast of Central America may prove separable from *M. birostris* of the

Atlantic by its color pattern". This statement suggests that even in 1953 there was an agreement that morphologically, mantas from different areas were very similar but the markings (colour patterns) were so distinctive they suggested different species, but no further investigation was made. The statement is particularly pertinent to the possible separation of Pacific *M. birostris* (*M. hamiltoni* according to Bigelow and Schroeder (1953)) and the north-west Atlantic variant of *M. birostris* which may be *M. giorna*. A method for distinguishing between *M. alfredi* and *M. birostris* by their markings is presented in Chapter 4. Over 99% of observations in this study were of *M. alfredi* with only 7 observations of the more oceanic *M. birostris* recorded. This result is important in providing evidence of sympatry between the two and that one species may be far more common in a particular location.

Unfortunately, the accepted view of a single species of *Manta* since Bigelow and Schroeder (1953) has meant that it is often unclear whether *M. birostris* (or *M.* sp. cf. *birostris*), *M. alfredi* or any combination were being referred to in a given reference. Where photographs or detailed observations were made available it was usually possible to identify the species by the colour patterns and morphology (e.g. the presence of the postdorsal protuberance). In addition all mantas greater than 5 m disc width (DW) were probably *M. birostris* (unless reported from the Caribbean). Published information on mantas was reviewed and the species separated where possible.

It appears that other charismatic megafauna have been subject to species investigation over the past decade, and the long-known single species separated into two or more species. The scalloped hammerhead shark *Sphyrna lewini* (Griffith and Smith 1834) is likely to consist of two species with the rarer cryptic species identified from DNA analysis (and not physical differences) and restricted to the western North Atlantic (Quattro et al. 2006). There appears to be at least three distinct species of orca (currently monospecific as *Orca orcinus* (Linnaeus 1758)) with the species separated by their movements and habitat (resident, transient or offshore), diet (fish and/or marine mammals) and DNA (Pitman and Ensor 2003; Taylor et al. 2008). Minke whales had already been separated into two species by the late 1990's: the common or northern minke whale *Balaenoptera acutorostrata* Lacépède 1804 and the Antarctic or southern minke whale *B. bonaerensis* Burmeister 1867. Additionally, it appears that there may be two or three subspecies of the northern minke whale (Rice 1998; Mead and Brownell 2005). It is pertinent that there is variation in the distribution of each species in all three examples.

2.4 Distribution

Mantas (genus *Manta*) are distributed circum-globally in tropical and warm temperate waters, occurring from 36ºS to 40ºN (Bigelow and Schroeder 1953; Smith and Smith 1966; Compagno et al. 1989; Michael 1993a; Last and Stevens 1994; Compagno 1999a; Homma et al. 1999). Mantas are reef-associated, oceanodromous and pelagic (Riede 2004; McEachran 2006), spending little time near the bottom. *M. alfredi* have been reliably reported from Australia, Hawaii, Yap, Maldives, Indonesia, Japan, South Africa, Mozambique (Whitley 1936; Homma et al. 1999; Anderson 2005; Acker 2006; Laros 2006; Dewar et al. 2008; Marshall et al. 2008) and French Polynesia (M. de Rosemont, pers. comm.). Sightings from the Atlantic are very rare. There were only two reports and photographs taken in the Canaries and the Cape Verde islands (Marshall et al. 2009) and historical reports and photos from the coast of Senegal (Cadenat 1958). There were no reports of *M. alfredi* from the western Atlantic. *M. birostris* is known from the Gulf of California, Revillagigedos Islands, Galapagos, Cocos, New Zealand, Brazil, Venezuela, NW Atlantic (Carolinas, Rhode Island) and the Gulf of Mexico (Bigelow and Schroeder 1953; Cervigon 1966; Duffy and Abbott 2003; Graham et al. 2008; Rubin et al. 2008; Luiz et al. 2009). *M. birostris* has also been reliably reported from Portugal (C. Ari pers. comm.), the Canary Islands and Madeira (P. Wirtz, pers. comm.) and throughout the Red Sea (pers. obs.). There have been occasional sightings from locations where *M. alfredi* is predominant including the Maldives (pers. obs.), India (Kunhipalu and Boopendranath 1981), Japan (Homma et al. 1999; Yano et al. 1999b), French Polynesia (Whitley 1936), Mozambique (Marshall et al. 2009) and Indonesia (R. Williams, pers. comm.).

Reports of *Manta* from the Caribbean, Bahamas, and the north-eastern American coastline include insufficient information to confidently identify species in view of the possibility of there being two similar species in the NW Atlantic. Reports suggested sightings of *M. birostris* from as far north as Rhode Island (Bigelow and Schroeder 1953) whilst *Manta* sp. cf. *birostris* was reported as far north as North Carolina (Coles 1916), throughout the Caribbean (Bigelow and Schroeder 1953; Smith-Vaniz et al. 1999; Marshall et al. 2009), and as far south as Venezuela (Notabartolo-Di-Sciara and Hillyer 1989) and appeared to occur in sympatry with *M. birostris* throughout its range. Marshall et al. (2009) suggested *M.* sp. cf. *birostris* replaces *M. alfredi* as the manta occupying shallower inshore waters in the Caribbean (Figure 2.1).

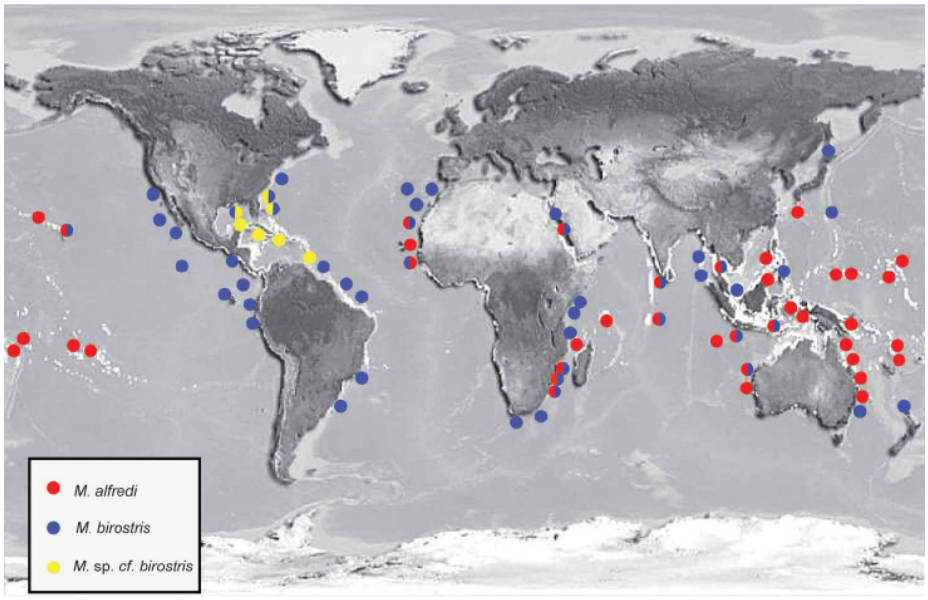

Figure 2.1 Worldwide distribution of *Manta* from preliminary analysis of 2231 images of over 100 aggregation sites and sighting records. The coloured dots represent confirmed sightings of the different manta species (see key on map for species code). Dots with two colours indicate regions where *M. alfredi* and *M. birostris* are reported in sympatry. From Marshall et al. (2009).

In summary, *Manta birostris* enjoys a wider distribution than *M. alfredi* with reports from sub-tropical and temperate waters, oceanic island groups and particularly offshore seamounts and pinnacles (Compagno 1999b). *Manta alfredi* is more commonly sighted inshore within a few kilometres of land along productive coastlines with consistent up-welling, and off of tropical island groups, atolls and cays. *Manta* sp. cf. *birostris* appears to be endemic to the Atlantic Ocean and Caribbean (Lesueur 1824; Mitchill 1824; Bancroft 1829; Coles 1916; Bigelow and Schroeder 1953). This Chapter focuses on *M. alfredi* and *M. birostris* as, due to difficulty in identification, very little is known of *Manta* sp. cf. *birostris* other than a physical description and its distribution.

Most records of depth range for mantas are from 0-40 m depth where they are easily observed by scuba divers (Henneman 2001; McEachran 2006). Investigations in the Yaeyama Islands, Japan, using ultrasonic telemetry showed *M. alfredi* swimming at 10-30 m depth during the day with occasional dives to 50 m, but moving offshore at night and swimming near the bottom at 50-200 m (Yano et al. 2000). Similar results were obtained in Hawaii (Clark 2008) and the Maldives (Guy Stevens, pers. comm.). Rubin et al. (2008) recorded night descents of satellite-tagged *M. birostris* to 200-450 m after spending daylight hours visiting shallower reefs. They were also recorded moving between the surface and 72-80 m depth during periods of travel in open ocean. Patokina

and Litvinov (2005) reported examining the stomach contents of two juvenile mantas (species unknown) trawled from a depth of 900 m. In summary, mantas of both species may be found in shallower waters during the day (0-80 m), they then may travel to offshore waters at night where *M. alfredi* was reported to dive to up to 200 m and *M. birostris* to 450 m. The maximum reported depth reached by mantas (species unknown) was 900 m.

2.5 Size

Records of mantas over 5 m disc width (DW) are likely to refer to *M. birostris* (or *Manta* sp. cf. *birostris*) and not *M. alfredi*. Last and Stevens (1994) report a maximum size of 9.1 m DW citing Bigelow and Schroeder (1953), however these authors reported a maximum size of 6.7 m (22 ft) based on a report from Bimini, Bahamas (La Gorce 1919), thus the Last and Stevens (1994) size appears unsupported. There is one measured and weighed record of 6.8 m DW and 2000 kg from Veraval, India (Kunhipalu and Boopendranath 1981). The 6.7 m manta from Bimini was measured but could not be weighed as the commercial scale available was only capable of weighing up to 3000 lb (1363 kg). Few specimens were actually measured due to the difficulty in handling such large animals and most records were 'sighted' or 'reported'. The 12 measured and weighed specimens are listed in Table 2.1 and used to establish a weight: DW relationship for the genus *Manta* (Figure 2.2).

Reference	Disc Width (cm)	Weight (kg)	Location
Bigelow & Schroeder (1953)	610	1588.5	Venezuela*
Kunhipalu and Boopendranath (1981)	680	2000	India*
Bigelow & Schroeder (1953)	427	764.8	Galapagos*
"	549	1047.8	Galapagos*
"	114	12.7	Galapagos*
Grant (1985)	430	750	Australia
"	150	9	Australia
Cervigon (1966)	600	1400	Venezuela*
Marshall (2008b)	132	15.1	Mozambique
"	223	71	South Africa
"	237	75	South Africa
"	223	71	South Africa

Table 2.1 This Table lists the size (disc width DW) and weight of *Manta* specimens reported in literature. The asterisks (*) indicate likely *Manta birostris* species; the others are likely or known *M. alfredi.*

That *M. alfredi* and *M. birostris* of about the same size (427-430 cm DW) are about the same weight suggests that growth characteristics are not radically different between the two species. These were all dead samples and elasmobranchs flatten/stretch when dead so the weight of a live animal cannot be directly predicted from these results.

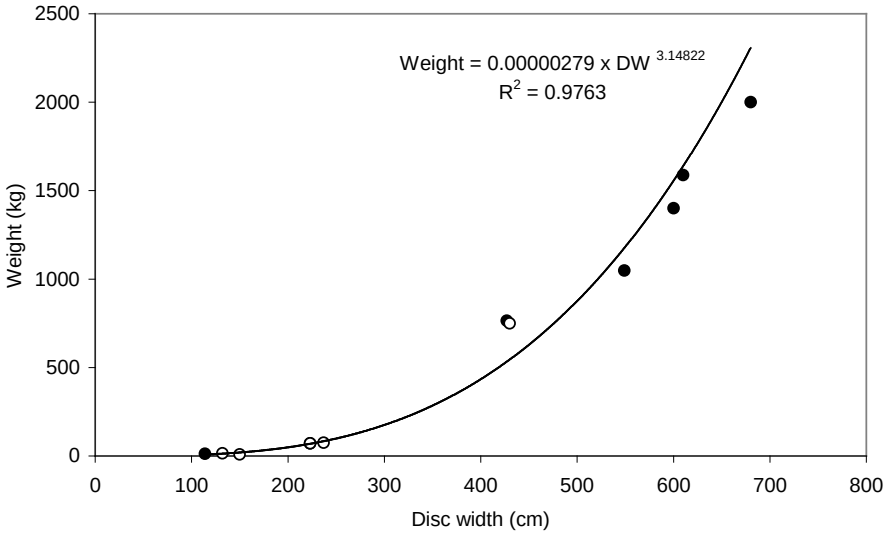

Figure 2.2 Graph of manta weight vs. disc width (DW) using data from Bigelow and Schroeder (1953), Cervigon (1966), Grant (1985), Kuhipalu and Boopendrath (1981) and Marshall et al. (2008). Filled circles are *M. birostris* and unfilled *M. alfredi*. Note the two almost overlapping points, despite being for different species, for DW 427-430 cm.

Despite the focus on maximum size there are some data on the typical size range at different locations. Care must be taken in assigning observations to either *M. birostris* or *M. alfredi.* Bill Acker (www.mantaray.com, originally accessed 20.11.2006) has been studying the mantas at Yap for many years and reported that the majority of mantas were between 2.67 and 4.67 m DW (8 -14 ft). Of mantas observed in Micronesia, Myers (1999) reported that they were seen with a DW 3 m or less. The Australian Museum Fish website stated that individuals to 4 m DW are common (McGrouther 2007) and Anderson (2005) gives a typical size of Maldives mantas at 3 m DW. Those four populations are known *M. alfredi*. The White et al. (2006) report of mobulid by-catch in Indonesia includes the biggest dataset of directly measured mantas (as opposed to those measured using estimation methods). 56 mantas were measured: the females ranged from 2.1 - 4.9 m DW (n = 33) and males 2.0 - 4.1 m DW (n = 21). The size range was suggestive of a *M. alfredi* population as no individuals exceeded 5 m DW. Marshall et al. (2009) reported a *M. alfredi* with an estimated DW of over 5.0 m in Mozambique and suggested the species has a maximum DW up to 5.50 m, however there was no

measured sample over 4.9 m. Marshall et al. (2009) reported dissected specimens of *Manta* sp. cf. *birostris* to 4.695 m DW. The largest field sighting of this species was over 6.00 m DW according to Coles (1916). The only sample examined by Marshall et al (2009) was an immature male 3.480 m DW, so the largest size achieved for this species is unknown.

In summary, *M. birostris* grows to a disc width of at least 6.8 m and weight of 2000 kg (Kunhipalu and Boopendranath 1981) whereas *M. alfredi* has been recorded to a maximum of 4.9 m DW (Marshall et al. 2009) which suggests an estimated weight (Figure 2.2) of 800 kg. The results of a study of mantas in the Maldives are presented in Chapter 4 and discussed further in Chapter 10.

2.6 Age

Little is known about the longevity of mantas. Mantas have been kept alive in aquaria for up to 13 years (Uchida et al. 2008) and were reported to live at least 20 years based on the personal observation of a divemaster who recorded a sub-adult female, species unknown (estimated to be c. 5 years old when first seen) over a period of 15 years (Homma et al. 1999). A manta was estimated to be 29+ years based on re-sightings of a female *M. alfredi* over a 29 year period in Hawaii (Clark 2008). The greatest age was deduced from a photograph of manta 209 L181 ‘Brenda’ from Lankan cleaning station (Maldives) in 1986 when she was already adult (assume at least 10 years old) and was therefore at least 36 years old when last recorded in February 2012. *Manta alfredi* thus appear to live at least 36 years. These examples were also important as they showed that individual mantas could be recognised from their markings for at least 25 years.

Age and growth studies in other elasmobranchs have been based on ring (or band) measurements of vertebrae sections (Branstetter 1987; Branstetter and Musick 1994; Piercy et al. 2007). Cailliet et al. (2006) conducted a review of 28 age and growth studies. Although the vertebral centra were the most common areas where calcified growth material were examined to measure age (23 studies), dorsal fin spines and caudal thorns were also useful. Some authors found an annual ring (band); while others concluded that ring formation was semi-annual, implying different growth rates between geographical areas. Moreover, in most of the studies the accuracy of the ring formation has yet to be validated. There was no study, nor even a report, of vertebral rings on a dead manta ray (Bigelow and Schroeder 1953; White et al. 2006; Marshall et al. 2008), although it is likely that they are present but unreported. A caudal thorn is

only present in *M. birostris* (Marshall et al. 2009). This subject presents an opportunity for study in an area where manta carcasses are available.

Examples of known longevity of other elasmobranchs in the wild include the common spiny dogfish *Squalus acanthias* Linnaeus 1758 achieving 65-70 years and recorded to a maximum length of 160 cm, and the bat eagle ray *Myliobatis californica* Gill 1865 with maximum age of 23 years and maximum DW of 180 cm (Hoenig and Gruber 1990). A positive relationship exists between body size and life span (Blueweiss et al. 1978) so comparing the known maximum DW and age of *M. californica* and *M. birostris* we might expect the maximum *Manta* age to exceed the known 36 years.

2.7 Anatomy

Until recently, the best description of manta anatomy (Figure 2.3) was by Bigelow and Schroeder (1953) which also included the best anatomical drawings available. Their study materials included a 3.48 m DW male from which the majority of anatomical notes were made plus a female and photographs of two other females. The male was identified as likely *Manta sp. cf. birostris* (Marshall et al. 2009) (A. Marshall pers. comm.). The other three samples of females might be *M. birostris* or M. sp. cf. birostris deduced from their sizes (DW 5.18 m, 6.45 m) and the post-dorsal protuberance on the smaller (4.57 m DW) sample (Figure 2.3, F and G). Only the location where they were obtained give clues to their species and is subjective. Bigelow and Schroeder (1953) did not make any comment about a possibility of mixed species amongst their samples and the similarity between *M. birostris* and *M.* sp. cf. *birostris* likely misled them to deduce that they were looking at a single species. Unfortunately they did not have a sample of *M. alfredi* to examine. Marshall et al. (2009) described anatomical variations and external physical characteristics of all three species, summarised in Table 2.2. Dorsal and ventral colouration was similar for all three species with both *M. birostris* and *M. alfredi* having melanistic (black) and leucistic (pale) forms. A study of the external markings of *M. birostris* and *M. alfredi* is presented in Chapter 4.

Physical characteristics shared by all three species and relevant to studies in later Chapters include the five gill openings and gill plates and cephalic fins. Ventral view (Figure 2.3 B, male, *M.* sp. cf. *birostris*) shows five gill openings on each side. Peculiar to mobulids is that each of the inner brachial openings through which the pharynx connects with the gill pouches is entirely surrounded by a single series of lamellae or gill plates that are thin, membranous and horny, with cartilaginous basal supports. There

may be 130-140 gill plates per arch in a manta of around 5 m DW. In *Manta* the plates are rod-like and adjacent plates are fused at their tips in a zigzag pattern to form a continuous grid or sieve on each of the faces (anterior and posterior) of each of the gill bearing arches. In *Mobula* the outer extremities of the extensions of the plates form rounded lobes which are separate from one another. The differences in the gill-plates structures are diagnostic in separating *Manta* and *Mobula*. A novel use of the intricate mesh of the brachial sieve is discussed in section 7.6.8. The gill plates serve the same purpose as the horny gill rakers of the basking shark *Cetorhinus maximus* (Gunnerus 1765) and the modified dermal denticles of the whale shark *Rhincodon typus* Smith 1828. Mobulid gill rakers are used in traditional Chinese medicine in the treatment of circulatory disorders and inflammation. Populations of mobulids in Indonesia and India are actively targeted to supply Chinese demand.

Characteristic	*Manta birostris*	*Manta* sp. cf. *birostris*	*Manta alfredi*
Disc width vs. disc length	2.2-2.3	Similar to *M. birostris*	2.2-2.4
Maximum DW	7000 mm (max. measured 6800 mm)	>6000 mm	5500 mm (max. measured 4900 mm)
Caudal spine	Reduced spine in calcified cartilaginous mass (post dorsal protuberance).	Reduced spine in calcified cartilaginous mass (post dorsal protuberance).	No caudal spine or cartilaginous mass.
Dermal denticles	Situated on long sagittally oriented raised ridges on dorsal and ventral surfaces.	Small knob like dermal denticles on both surfaces, non-overlapping but densely and non-uniformly distributed.	Small knob like dermal denticles evenly distributed on both surfaces.
Dentition	Small cusped teeth in lower jaw in 12-16 rows. Total tooth counts 3000-4000. Upper jaw contains at least two rows of enlarged denticles.	Tooth band lower jaw only containing 9-11 rows of small cusped teeth.	Small cusped teeth roughly in 6-8 rows. Total tooth count 900-1500. Top jaw lacks enlarged denticles.
Shoulder patches	Triangular shaped pale patches on either side of dark midline.	Absent.	Pale patches emanating anteriorly from spiracle and curving medially
Ventral markings	No markings between gill-slits. V shaped margin along posterior edges of pectoral fins.	Spots or melanistic patches in posterior ventral area only.	Markings present between gill-slits. Spots may occur across most of ventral surface.

Table 2.2 The main external characteristics to distinguishing *Manta* species (Marshall et al. 2009). Note maximum DW sizes are all proposed, not measured, with known sizes in brackets.

The spiracles are small, and like other batoids, mobulids take in water for respiration chiefly through their mouths. A discussion of how water intake for respiration may affect behaviour and how different behaviour states may be deduced from the cephalic fin positions and degree of openness of the mouth can be found in section 7.6.8.

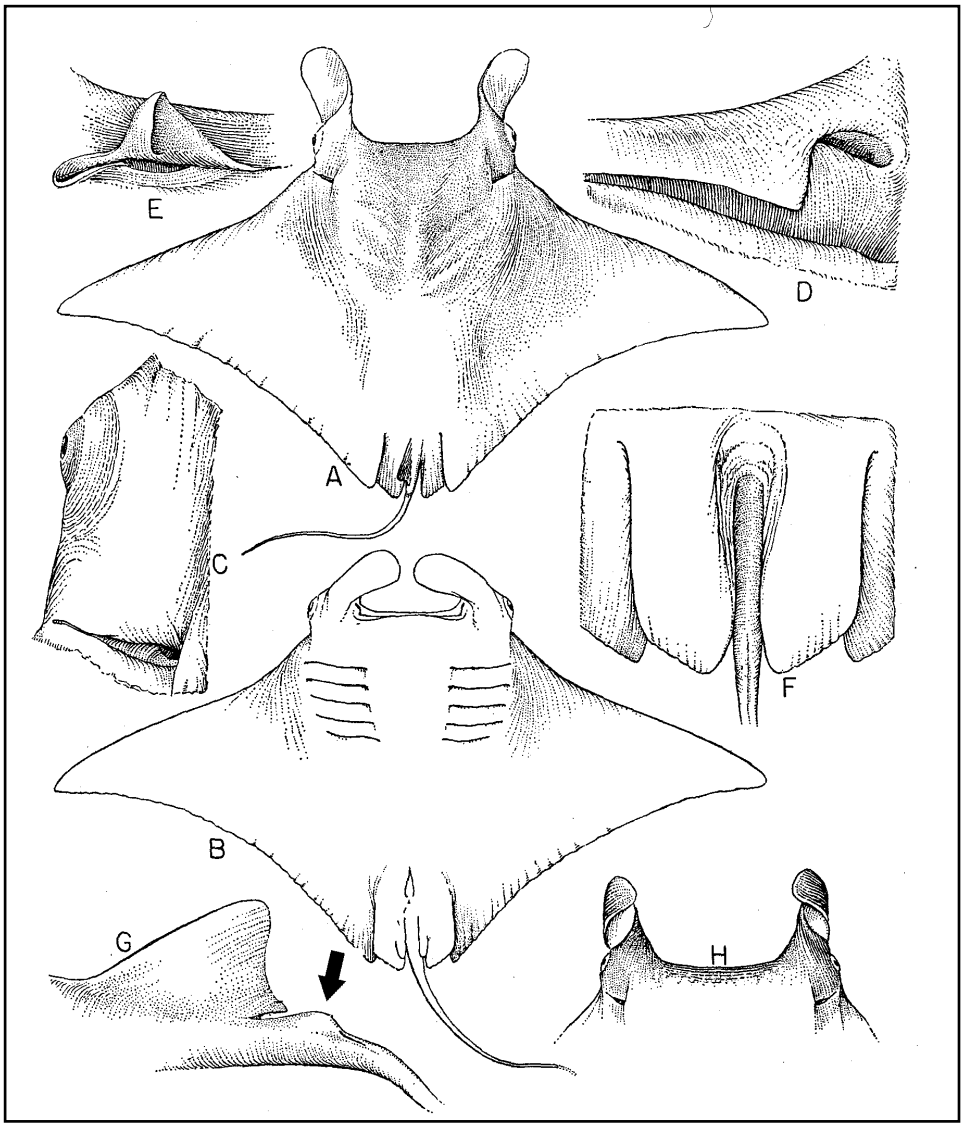

Figure 2.3 Plate from *Fishes of the Western North Atlantic* (Bigelow and Schroeder 1953). These are among the best drawings of manta external morphology available. **A:** Dorsal view of juvenile male (*M.* sp. cf. *birostris*) approx. 3.48 m DW. **B**: Ventral view of same. **C**: Detail of left eye and spiracle from A. **D**: Left nostril and outer part of nasal curtain. **E**: Outer part of right nasal curtain rolled forward to show transverse fold on internal surface. **F**: Pelvic fins of female (4.57 m DW). **G**: Dorsal fin and base of tail of same. Note the post-dorsal protuberance (arrowed) found in both *M. birostris and M.* sp. cf. *birostris*. H. Front of head of female (6.45 m DW) taken off New Jersey, to show cephalic fins coiled in swimming position.

The mouth occupies about 63% of the extreme breadth of the head. The lower jaws are weakly convex forward with a slightly overlapping upper jaw. Teeth rows are used to distinguish *Manta* species and a lack of teeth in the upper jaw defines *Manta alfredi* (Marshall et al. 2009) unless the sample was obtained in the north-west Atlantic. Whitley (1936) was the first to use dentition as a character to define species. He described two subfamilies of "Ceratopteridae (Mantidae)". Ceratopterinae, with teeth on lower jaw (which included a species he described as *Daemomanta alfredi*), and Indomatinae, with teeth in both jaws. For the latter subfamily no description was given nor examples from the list of possible species known at the time.

The cephalic fins are about half as broad at base as long. The transverse axes are nearly vertical on the sides of the head, and upper margins about level with midpoint of the front of head. The lower edge is thinner than the upper edge which is fleshy and rounded. The tips are broadly rounded. The cephalic fins are rolled spirally with lower margins outward (Figure 2.3 H) when swimming, or flattened vertically when feeding and the outer ends are capable of being incurved so that the tips almost meet. The manta's cephalic fins have evolved specifically for this purpose and differentiate them from other members of Mobulinae (Devil Rays), which all have fixed horns, or minimally moveable fins (Notarbartolo-Di-Sciara 1987). Section 7.6.8 reviews cephalic fin positions.

2.8 Reproduction and reproductive behaviour

Elasmobranch reproduction was summarised by Carrier et al. (2004) and Hamlett (2005). All elasmobranchs have internal fertilization and the embryos are retained for a period of time. Elasmobranchs can be divided into two groups: oviparous (egg-laying) and viviparous (live-bearing) forms. Viviparous forms may be further subdivided into lecithotrophic (yolk dependent), oophagic (egg-eating), and matrotrophic (foetal development is augmented by maternal input of nutrients). Aplacental viviparous forms do not have a placental connection between mother and offspring; in the past this was called ovoviviparity. Histotrophy is the type of matrotrophy common in Myliobatiformes. The female produces a lipid-rich histotroph (or "uterine milk") secreted by long villi called trophonemata (Alcock 1892) on the uterine lining and ingested by the embryos (Amoroso 1960). This form of matrotrophy was confirmed in *Manta birostris* by White et al. (2006).

Reproductive cycles in elasmobranchs are poorly understood. There has been no specific study of mantas. The reproductive cycle encompasses the ovarian cycle and gestation period and the two processes may run concurrently or consecutively, e.g. in some species of elasmobranch the oocyte may take 12 months to develop, conception may then occur, followed by a 12 month period of gestation. This cycle is biennial. In other species the development of the oocyte in the ovary occurs at the same time as the development of the embryo in the uteri. This concurrent reproductive cycle could allow for an annual cycle if the gestation period is less than a year (Castro 1996). All reports of births and investigations of the uteri of pregnant female mantas indicated the presence of a single embryo (Bigelow and Schroeder 1953; Homma et al. 1999; Berriman 2007; Uchida et al. 2008).

Homma et al. (1999) postulated that “pregnancy may last up to two or three years” in mantas based on a female being known to give birth (not observed) three times in a six to seven year period. The recent births of two (1.82 m and 1.92 m DW) *M. alfredi* in captivity 368 and 374 days (respectively) after mating (Matsumoto and Uchida 2008; Uchida et al. 2008) indicated a gestation period of about one year. Mating and successful conception occurred in the same female immediately after parturition suggesting a cycle of 12-13 months (Uchida et al. 2008). In mammals, environmental conditions during pregnancy may determine birth weight, and the length of gestation and size of offspring may depend on the nutritional status of the mother (McLaren 1965; Mellor 1983). It has been established that the placenta is important in the regulation of nutrition to mammalian foetuses (Vaughan et al. 2012). There does not appear to be any published data on elasmobranchs however it is likely that environmental effects would have some impact on manta offspring due to them being fed a histotroph produced by the mother. In a situation where food was scarce, the mother would be in a poor nutritional state so her offspring would also be malnourished, and vice-versa. It is possible that the 12 to 13 month pregnancies and size of offspring observed in captive *A. alfredi* were atypical, but as the two pregnancies were similar in length and the offspring were relative large it is likely that they were representative of full-term pregnancies. There are reports of free-swimming mantas (species unknown) as small as 1.02 m DW and 1.22 m DW (Homma et al. 1999; Patokina and Litvinov 2005), whereas Bigelow and Schroeder (1953) recorded a *M. birostris* embryo 1.14 m DW (45") and a free swimming manta (species unknown) of 1.22 m DW (4 feet). Overall

these suggest that birth sizes range from 1 m to almost 2 m DW in *Manta*. The frequency of pregnancies and length of cycles are investigated in Chapter 10.

Frisk et al. (2001) offered a formula for the relationship of age at maturity in sharks, skates and rays based on maximum natural age; where T_{mat} is age at maturity and T_{max} is longest natural age based on a sample of 35 elasmobranch species.

$$T_{mat} = 7.20 \ln (T_{max})-12.68$$

Homma et al. (1999) postulated the range of age of sexual maturity in manta to be 8-13 years, although there was no evidence supporting this range. Using 36 years minimum T_{max} (based on the known 36 years) suggests the minimum age of maturity in female *M. alfredi* to be 13 years.

Parturition was recently recorded from a 4.0 m DW *M. alfredi* in captivity. The mother was captured as a juvenile and held in captivity for around 10 years before she mated and was estimated to be around 13 years old at the time of mating (Uchida et al. 2008). This observation supports the calculated age above. The 1.92m DW neonate had both wings folded over its dorsal surface (Figure 2.4 A); these stayed tightly furled for 10 s after birth, then opened to a vertical position. For the next 30 s the manta attempted to swim with the wings moving from the half way to the closed position (Figures 2.4 B and C) before the wings were completely unfurled to allow proper swimming movements. Subsequently, the neonate was able to use its wings fully and swam energetically. This observation suggested that a neonate manta would be at significant risk from a large predator during the first few minutes after birth.

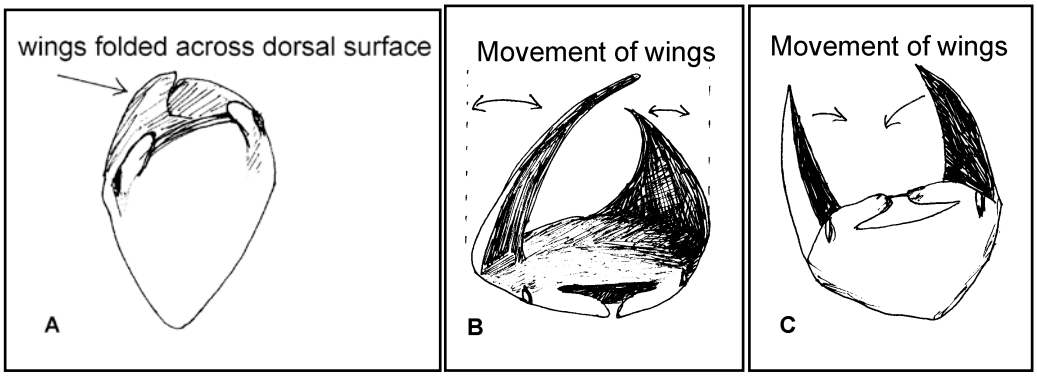

Figure 2.4 Drawing of (A) 1.92 m DW *Manta alfredi* at birth showing wings tightly closed across dorsal surface. B: at 10 seconds, neonate manta attempted to flap wings but had very restricted movement. C: at 15 to 40 seconds after birth the manta was able to swim with wings in up-to-closed position but sank towards the bottom twice. This sequence was based on a video of a birth accompanying Berriman (2007).

2.8.1 *Courting and mating behaviour*

There was only a single report of manta mating behaviour. Yano et al. (1999) observed *M. birostris* (species deduced from photographs) mating in the Ogasawara Islands, Japan and described males chasing females prior to copulation. They described the act of copulation in detail, reporting that the male clasped the left wing tip of the female to maintain position and that mating was abdomen to abdomen. They speculated that the chasing may be due to the female excreting a chemical stimulus into the water as well, and the chase-follow behaviour may be a pre-copulatory releaser. In addition for the male needing to attach to the female during copulation, Springer (1960) hypothesized that elasmobranch biting behaviour was a pre-copulatory releaser to invoke female acquiescence, a hypothesis supported by several observations of biting during courtship in a number of sharks: *Heterodontus francisci* (Girard 1855), *Orectolobus japonicus* Regan 1906, *Ginglymostoma cirratum* (Bonnaterre 1788), *Carcharias taurus* (Rafinesque 1810), *Triakis scyllium* Muller and Henle 1939, *Triaenodon obesus* (Rüppell 1837), and *Prionace glauca* (Linnaeus 1758), and rays: *Raja eglanteria* Bosc 1800 and *Aetobatus narinari* (Euphrasen 1790) (Libby and Gilbert 1960; Dempster and Herald 1961; Stevens 1974; Klimley 1980; Tricas 1980; Uchida et al. 1990; Gordon 1993; Hagiwara 1993; Carrier et al. 1994; Yano 1998). Observed biting behaviour may have a similar function in courtship of manta rays and this is discussed further in section 10.5.3. Yano et al. (1999) suggested that there was a specific mating season and reported that *Manta birostris* mated primarily in July-August in the Ogasawara Islands. The courtship and mating behaviour of the *M. alfredi* in the Maldives is discussed in Chapter 10.

2.8.2 *Size at sexual maturity*

There are few observations on size at sexual maturity. Bigelow and Schroeder (1953) reported pregnant female mantas of 4.3-4.6 m DW (species and location unknown), whereas they found the 3.48 m DW male (*M.* sp. cf. *birostris* sample obtained in Bahamas) to be immature. Both Bigelow and Schroeder (1953) and White et al. (2006) suggest males mature at a smaller size than females which is common in elasmobranch species. Yano et al. (1999b) observed successful mating between a 5 m DW female and two approximately 4 m DW male *M. birostris*. White et al. (2006) described samples from throughout Indonesia that have been identified as *M. alfredi.* All mature males were greater than 3.7 m DW (n = 11), and mature females greater than 4.1 m DW (n = 6). The mating *M. alfredi* pair reported by Uchida et al. (2008) were 3.6 m and 4.1 m

DW respectively for the male and female. Individuals from the *M. alfredi* populations observed in Yap (Acker 2006), Big Island, Hawaii (T. Clark, pers. comm.) and the Maldives rarely exceed 4 m DW but are observed mating and pregnant. This study investigates size of sexually mature *Manta alfredi*, reported in Chapter 10.

2.9 Migration behaviour

Homma et al. (1999) described various migration behaviours. They described movements from surface feeding locations to cleaning stations during the day and annual migration of some mantas from Yaeyama islands to Kerama Island, a distance of 350 km (the species performing the migration was recently confirmed as *M. alfredi* (Kashiwagi et al. 2010)). They also noted that mantas in Yap (*M. alfredi*) appeared confined to the islands. According to information on the www.mantaray.com website (B. Acker, access date 10.10.2006), some individual mantas migrated from east to west sites, a distance of less than 10 km, from the summer to winter months, respectively. Results from telemetry studies in Hawaii indicated that *M. alfredi* were usually found within a home range of around 10 km (Clark 2004), and that the pattern of daily movement included travelling to shallow reef cleaning stations during the day and to offshore deep water to feed at night (Clark 2008). Acoustic tagging research of mantas in the Komodo Marine Park indicated that tagged mantas used the park all year round with 5 out of 7 of the animals (with the longest records) spending 90% of their time at the location where tagged. Overall 81% of visits were to the same site as where tagged. Where movement occurred (between feeding and cleaning sites, or seasonal movements) the distances travelled were typically <20 km. The authors suggested that there were residency patterns within the park and the mantas exhibited considerable site fidelity. Some mantas were not recorded after a short period but it was not known whether they lost their tags or moved away (Dewar et al. 2008). The species was recorded as *M. birostris* before the species were separated, but as *M. alfredi is* predominant in this locality it was assumed that the study was of *M. alfredi*. In the Maldives, a manta (*M. alfredi*) was re-sighted in the same monsoon period the following year (site not recorded), and it was suggested that the rays migrated from one plankton-rich side of the atoll to the other and back with the seasonally changing monsoons as they were absent from the windward sides during the relevant season (Anderson 1996).

M. birostris in the Revillagigedos islands are known to migrate between groups of islands 613 km apart and have been tracked travelling distances of up to 2240 km in 12 days (Rubin et al. 2008). The mantas have been studied in this location since 1978, however they have been re-sighted relatively infrequently (R. Rubin and K. Kumli, pers. comm.) with gaps of many years between sightings. By 2009 only 108 of 321 (33.6%) mantas identified had been re-sighted (K. Kumli, pers. comm.).

In summary, *M. alfredi* in Yap, Hawaii and Komodo were reported occupying a relatively small area, travelling distances of around 20 km between sites. They might be seen within this area year-round, but may attend different cleaning and feeding areas depending on the season. Individual *M. alfredi* in Yaeyama and the Maldives appear to migrate with seasonal changes, travelling distances of up to several hundreds of kilometres each year. Individual *M. birostris* in the East Pacific appear to travel much greater distances. The roaming behaviour of mantas appears to fall into three main categories. 1) Resident: these mantas have a relatively small home range (tens of kilometres) and are observed in specific areas year round. Examples are Hawaii, Komodo and Yap populations (Homma et al. 1999; Clark 2008; Dewar et al. 2008; Deakos et al. 2011). 2) Migratory: these mantas are known to travel distances of up to several hundreds of kilometres each year, following cyclical patterns of weather, but return to the same area year after year after completing the migration. This movement pattern was described by Homma et al. (1999) (southern Japan) and alluded to by Anderson (1996) (Maldives). Mantas showing resident and migratory behaviour are sometimes referred to as ‘reef’ or ‘inshore’, due to their association with coral reefs and this name has been adopted by some scientists as a common name for *M. alfredi*. 3) Oceanic: describes the behaviour of the giant manta *M. birostris*. The species appears to have attracted the additional common names of ‘pelagic’ or ‘oceanic’ manta. Although juveniles are seen in inshore waters (Graham et al. 2008), adults are known to spend considerable time in the oceanic province and are occasionally seen at remote oceanic islands (Cocos, Galapagos and Revillagigedos in the East Pacific (Rubin et al. 2008) (Karey Kumli, pers. comm.), St Pauls Rocks in the central Atlantic (Luiz et al. 2009)) or making occasional visits to sites where *M. alfredi* predominate.

The movements, migrations and the common names of the different species are discussed in Chapter 5, following the results of the investigation on the movements of individual *M. alfredi* in the Maldives.

Chapter 3. Geographical overview of the Maldives, study area and survey sites, and an introduction to survey and data collection methods

3.1 Abstract

The survey sites in this study were principally areas of reef containing cleaning stations which are visited by scuba divers during the relevant monsoon to observe mantas. The sites were typically located on the leeside margins of the atolls in areas where plankton productivity was high. It appears that the effects of reef structure and water movement combine in the Maldives to create areas of upwelling of nutrients and areas with high plankton concentrations (including zooplankton which is the food of mantas). Mantas would be expected to accumulate to feed in these areas, and also be attracted to nearby cleaning stations where they can be studied. Reef structures, weather, currents, tides and other less regular events (resort construction, El Niño Southern Oscillation) may affect the ecology of mantas in the Maldives. This study includes results from surveys conducted throughout the Maldives. These survey sites are introduced and more detailed information is provided on the seven main sites accounting for around 97% of manta sightings. The main observation strategies and types of surveys used in the study are also described.

3.2 Introduction

Since tourism was introduced to the Maldives in 1972, visits to areas of reef where mantas were observed being-cleaned (cleaning stations (Feder 1966)) were carried out as part of a diving tourist itinerary. The cleaning stations were initially discovered by divers with assistance from local fishermen who had regularly observed mantas in specific locations and passed intelligence onto the dive centre staff. The cleaning stations were areas of reef with one or more areas of colonies of cleaner fish. Sometimes the colonies were clustered on a single bommie, and at other locations the cleaner fish were distributed along the reef. These locations quickly became established “manta points”; local terminology for a dive site where manta encounters could be anticipated. Many of the selected survey sites in this study were such established “manta points” (Lankan Reef, Madivaru and Kalhahandi Huraa) but several were discovered during the course of this study (Table Thila and Desperation Thila,) (Table 3.2, Figure 3.8). There was little information published on manta cleaning stations in the Maldives apart from various recreational diver guides to the Maldives (Harwood and Bryning 1998; Godfrey 2006), which only contain a short paragraph (c. 120 words) on each site. The sites are

influenced by monsoon wind direction, currents and reef geomorphology, all of which appear to have a significant influence on the availability of food (zooplankton) for mantas. It was assumed by other researchers of mantas that the distribution of mantas was influenced by the distribution of suitable planktonic food (Notabartolo-Di-Sciara and Hillyer 1989; Homma et al. 1999; Dewar et al. 2008; Luiz et al. 2009). This link was recently established in the Maldives by Anderson et al. (2011). Thus, an introduction to the geographical, tidal and weather influences on water flow is relevant.

This Chapter covers several topics. It summarises the geography of the study area and reviews weather patterns, currents and tides with respect to manta ecology. Two events having an effect on Maldives reefs are briefly reviewed for their effect on manta ecology (the 1998 El Niño Southern Oscillation (ENSO) and the development of Paradise Resort- Lankanfinolhu). Secondly, a detailed description of the seven primary survey sites located within North Male and Ari atolls is provided. Thirdly, the main observation and survey methods are described. These were the generic methods used to perform surveys and gather data. Specific methods required for particular studies are referred to in the relevant Chapters. The detailed information was intended to provide future researchers with sufficient background information on reef conditions in the Maldives to 1) plan safe survey dives, and 2) replicate surveys.

3.3 Geography of the Maldives: geology and reef formation

The Maldives islands are a series of coral atolls, cays and faros (small ring reefs within an atoll) (Darwin 1842; Scheer 1972) developed on the Lakshadweep-Chagos ridge, which was generated by the northern drift of the Indian plate over the Réunion hotspot approximately 55 Myr ago (Aubert and Droxler 1992). The word *atoll* comes from the *Dhivehi* word *atholhu* (first recorded in English use in 1625 as *atollon*). The word atoll was used by Darwin (1842) and defined as “circular groups of coral islets”. The Maldives would be considered true atolls based on the modern definitions of “an annular reef enclosing a lagoon in which there are no promontories other than reefs” and “islets composed of reef detritus” (McNeil 1954) and “a ring-shaped ribbon reef enclosing a lagoon” (Fairbridge 1950).

The chain of atolls extending from the Lakshadweep through the Maldives and south-west to Chagos is the largest and most extensive on the planet. Holocene reef development has resulted in a number of large atolls of 20-150 km diameter. The lagoon

depths vary from 30-100 m, with great variation in depth even in neighbouring atolls. Between Noonu (North Miladhunmadulhu) and Thaa (Kolhumadulu) atolls the chain splits into two creating a parallel chain of atolls (Figure 1.1). The atoll sides are steep, caused by reefs growing under moderate energy conditions. The distribution of lagoon floor depths probably represents Holocene reef growth (over the past 8,000 years) on a surface previously eroded flat by a sea-level drop (Kench et al. 2009). There is considerable variation between the north and south of the Maldives chain in the structure and development of the atolls (Woodroffe 1992). In the north and centre they are broad banks, discontinuously fringed by reefs with small islands and many faros and the atoll fringing reef often has faro-like structure with an enclosed lagoon (Figure 3.1).

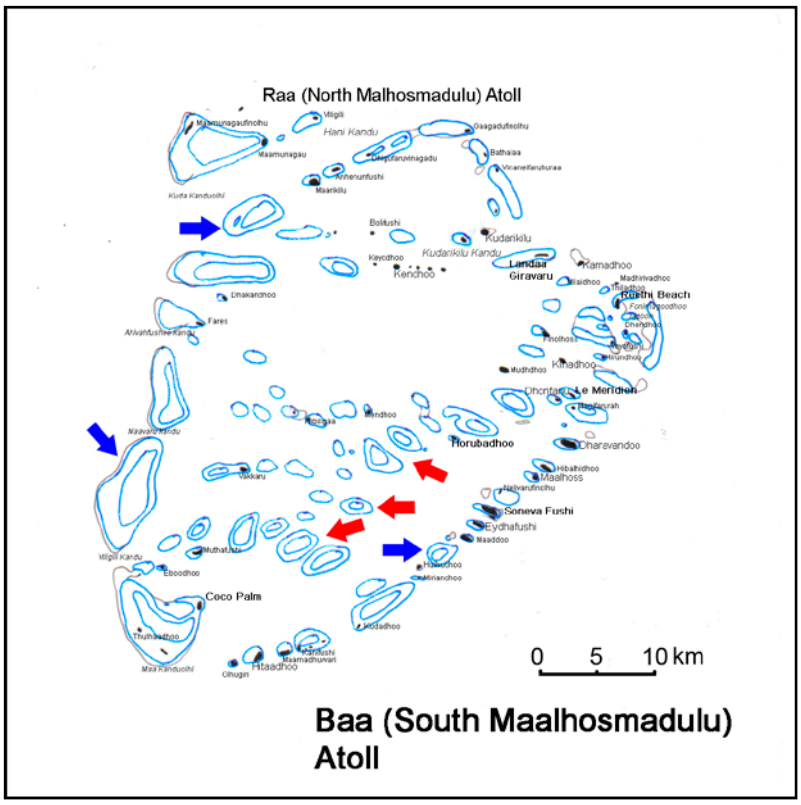

Figure 3.1 Baa atoll has many faros. Some typical examples are indicated by red arrows in south-central area of atoll. Faro-like fringing reefs are indicated by blue arrows and are shallow lagoons on edges of the atoll entirely surrounded by shallow reef.

These faros or ring reefs range in diameter from 10's to 100's of metres. Mantas, particularly juveniles, are often observed inside or nearby faros. Including, and south of Felidhoo atoll, the rims of the atolls are more complete (i.e. they have fewer channel breaks) and faros are absent. Islands are almost entirely made of sand which accumulates in sheltered shallow coral areas. Growing corals and calcium-depositing algae bind the coral/sand mass together and fresh coral growth on top continues the process. The opposing monsoon seasons cause periods of erosion and growth in shallow coral areas allowing equal upwards accretion, and this may be the cause of the faros'

growth (McClanahan et al. 2000). The more recent reef growth is built upon a limestone base approximately 2000 m thick. To the east and west of the archipelago depths to 2000 m can be found 1-8 km offshore. Some of the external fringing reefs on the outside of atolls drop off almost vertically (e.g. 0-600 m within 200 m of the shoreline in Rasdhoo atoll). Others drop off gradually to about 200 m several hundred metres off-shore, and then drop off steeply to the ocean floor (Purdy and Bertram 1993). Sea depth between western and eastern atolls in the central double-chain is approximately 350 m. Depth in the main channels running east-west between atolls is up to 2000 m (Godfrey 2004) (Figure 1.1).

The slope outside of the ocean-facing fringing reefs on the east and west sides of some atolls (particularly west Ari and east North Male atolls) (Purdy and Bertram 1993) creates a shallow step where plankton washed out of the atoll by the seasonal oceanic currents could accumulate, rather than being carried out to sea. The step might also capture nutrient upwelling (Figure 3.2). Possible mechanisms for the trapping of plankton and nutrients are discussed in the next section.

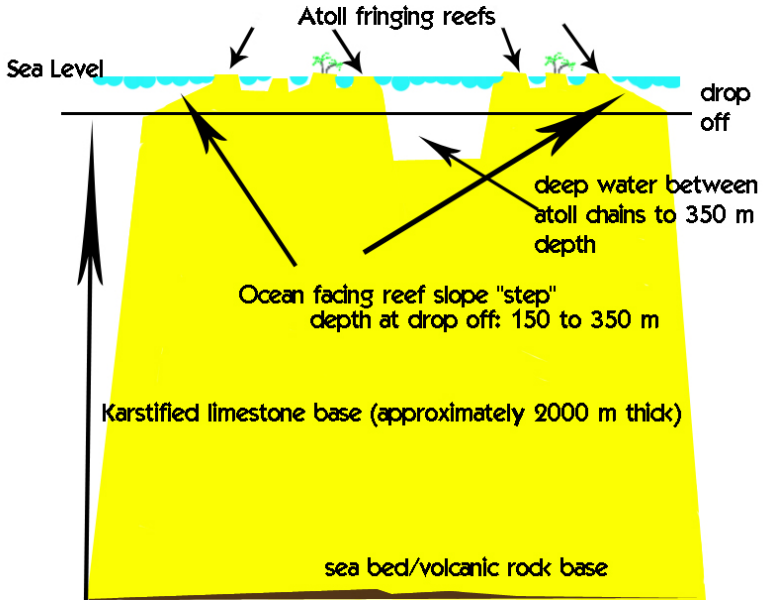

Figure 3.2 Cross-section of the Maldives plateau (central atolls), to contrast the height of main plateau and depths of seawater to east and west of chain (2000 m+) with the relatively shallow depth (350 m) between chains. Between fringing reefs and islands within the atolls depths are between 30 and 100 m. Some ocean-facing reefs slope gently to 150-350 m, creating a step, before dropping off steeply (Purdy and Bertram 1993).

Although this phenomenon has not been studied scientifically, the majority of known manta cleaning stations (18 out of 27 sites listed in Table 3.2) occur on the shallow reef adjacent to where these steps are known to occur (including Lankan Reef, Kani, Table Thila, Emas, Himendhoo, Madivaru) so this feature of the outer reef slope appears to be conducive to the presence of mantas in nearby shallow reefs.

3.4 Weather patterns, current and tides

The climate is tropical-oceanic with very little temperature variation either during the day or through the year (Gardiner 1903; Harwood and Bryning 1998).The Maldives are in the monsoon belt of the northern Indian Ocean, and experience a dry north-east monsoon locally known as *Iruvai* (east) and a wet south-west monsoon known as *Hulhangu* (west). The dry north-east monsoon lasts from mid-December to the end of April (average 75 mm rainfall per month and average monthly sunshine 256 hours) and the wetter south-west monsoon lasts from May to November (215 mm average rainfall per month and 208 hours sunshine per month). The months of April and November are change-over months and winds are typically small and variable in both. The Maldives are sometimes affected by tropical cyclones passing through the Bay of Bengal, particularly in April/May and October/November but the Maldives is outside the cyclone belt, being so close to the equator and is rarely exposed to winds in excess of 80 km/h.

The monsoonal weather pattern has a strong influence on the currents in the Maldives. During the north-east monsoon oceanic currents are driven through the atolls from the north-east and conversely oceanic currents are driven through from the west or south-west during the south-west monsoon. It can take several weeks after the change of wind direction in either monsoon for the oceanic currents to change direction and flow in the same direction as the wind. This can make sea conditions rough during the changeover months in periods of high winds. Conditions vary from year to year and the change of monsoons may be early or late and accompanied by rapid reversal of oceanic flow or a delayed reversal. Once established the oceanic currents seem little affected by tides. During settled periods the windward side atoll rim channels have inward flow for many weeks at a time causing clear oceanic water to flood into the atolls and displace water enriched with plankton to the leeward side of the atolls. This creates a great disparity between conditions on opposite sides of the atolls in terms of levels of nutrients and presence of plankton, but as the prevailing conditions reverse with the monsoon change,

coral growth on both sides of the atolls is similar. My personal observation of recovery since the 1998 ENSO related mass-bleaching event is that reefs on the west chain of atolls have higher growth of tabulate *Acropora*.

Planktivorous mantas would be expected to accumulate at areas of greatest plankton concentration. The mantas would follow their food source as is observed in other wide-ranging marine animals (Sims et al. 2000; Nelson and Eckert 2007; Domeier and Nasby-Lucas 2008; Moore et al. 2010). Mantas are more commonly sighted on the lee side of the atolls during each monsoon (Anderson et al. 2011), and thus appear to migrate at the monsoon switch-over. Manta migration and movements are discussed in Chapter 5. The phenomenon of increased plankton on the lee side was discussed by Sheppard (2000). 'Haloes' of high phytoplankton are observed around oceanic atolls and have been assumed to represent 'leaking' of nutrients from the reefs and associated systems into the nutrient poor ocean. The halos may also be caused by mild but sustained upwelling. Upwelling brings nutrients from deep water into shallow water where it becomes accessible to phytoplankton which then thrives. The upwelling is a result of the interaction between ocean circulation and island topography caused by the island mass effect (Doty and Oguri 1956; Gilmartin and Revelante 1974; Sengupta and Desa 2001). In the Maldives the upwelling would occur on the leeside of the atolls (upwelling nutrient-rich water from the deep water outside of the atolls (see Figure 3.2). Phytoplankton supports the rest of the marine food chain leading to increased productivity in the area of the halos. A westward plume of high chlorophyll-a (chl-a) was described occurring in the Maldives during January to March (the peak of the NE monsoon), and an eastward plume from October to December (the end of the SW monsoon) (Anderson et al. 2011). These correlate with sightings of mantas on the plankton-enriched sides. High chl-a indicates the presence of high levels of phytoplankton on which zooplankton (the food of mantas) feed. This phenomenon is being investigated at the time of writing and recent chl-a concentration maps (Figure 3.3) show the plumes graphically (Anderson et al. 2011). This supports the contention that there is greater productivity in the lee of the prevailing monsoon winds, and this greater productivity might attract mantas.

Tides are mixed, mainly semi-diurnal and have small ranges averaging 70 cm in the northern and central atolls and 100 cm in the south (Stoddart 1971; Couper 1983). Surface water temperatures vary from 25 to 30ºC although extremes of 23ºC and 31ºC

have been experienced at the season changeovers. Strong currents and winds at the start of both monsoons bring colder water to windward sites, whilst calm, low current conditions (particularly at the end of the NE monsoon) allow surface waters to increase in temperature (NOAA Coralreefwatch Pathfinder sea surface temperatures). An extended calm period at the end of the NE monsoon of 1998 may have contributed to the extreme warming of sea surface temperatures in the Maldives that year (Edwards et al. 2001), reducing water mixing and increasing light-stress on corals and thus exacerbating bleaching.

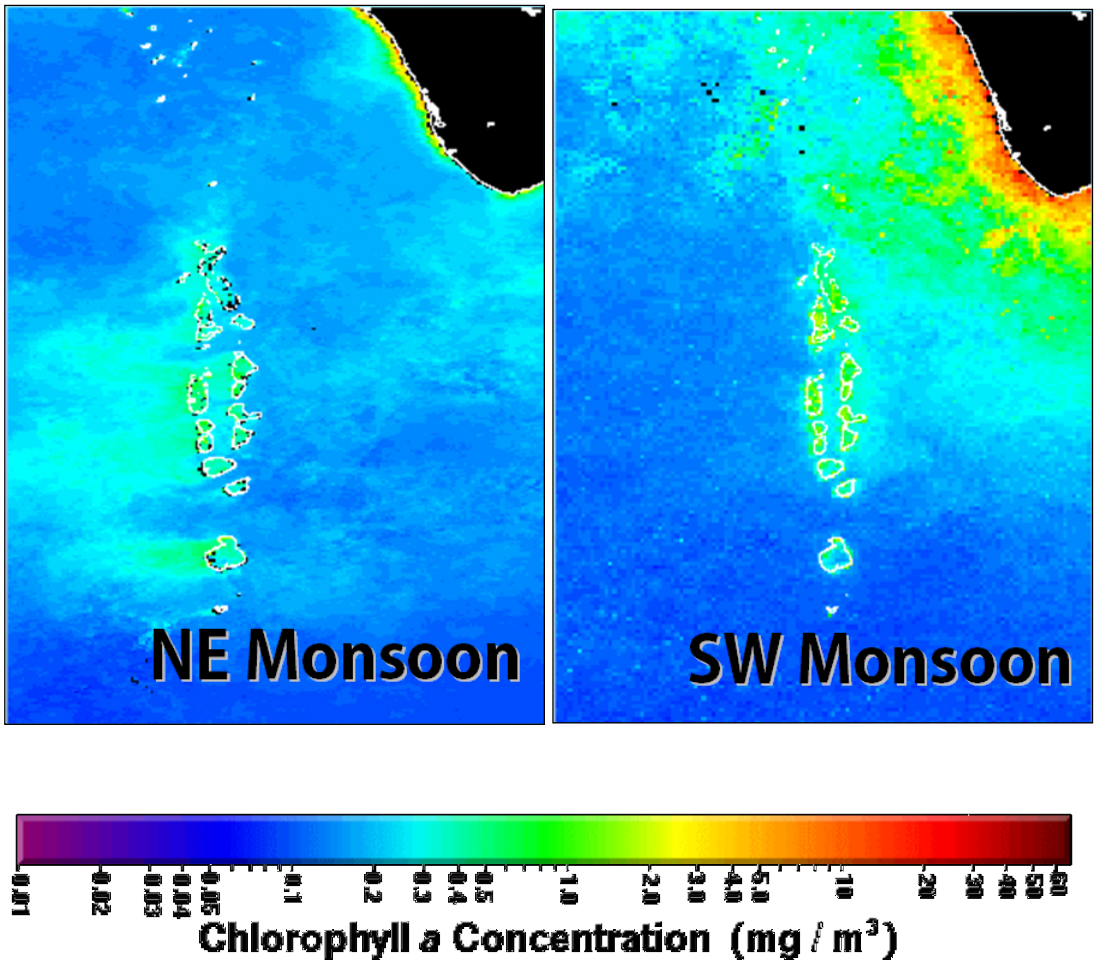

Figure 3.3 False colour images showing chlorophyll-a concentrations, which were estimated from remote sensing data obtained from NASA's ocean colour satellite SeaWiFS. The datasets used for the study were 9 km, monthly Global Area Coverage (GAC) standard mapped images. Monthly fields of chl-a were binned to generate seasonal maps of chl-a for the NE monsoon (Dec. 2006 to Mar. 2007), and SW monsoon (June to Sept. 2000). The images were prepared by Helga do Rosario Gomes and Joaquim Goes (Anderson et al. 2011). The images show the outline of the Maldives (centre left) and the SW coast of India (top right).

The effect of tides are moderated or increased by oceanic water flow caused by the monsoon winds. Windward facing reefs are subject to almost continual in-flow of oceanic water whilst leeward channels have stronger outflows during falling tides and minimal in-flow during rising tides. Figures 3.4 and 3.5 illustrate the NE monsoon situation.

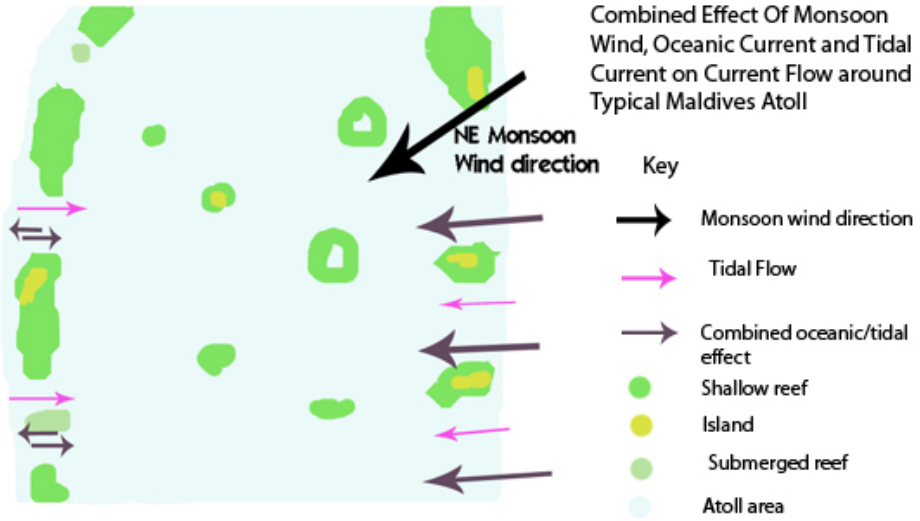

Figure 3.4 Diagram of part of an atoll to show effects of combination of monsoon wind and ocean current with tidal flow on a rising tide. During the NE monsoon the ocean current flow is mainly with the wind direction (E or NE) on a rising tide the effects combine on the windward side to create strong inflow of water but cancel out or reduce inflow on the west side preventing an inwards flow of oceanic water on the west side.

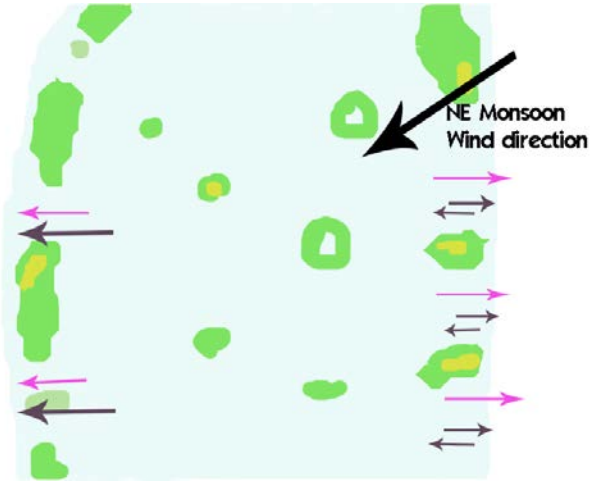

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Figure 3.5 Diagram of part of an atoll to show the combined effects of monsoon wind and ocean current with tidal flow on a falling tide. A cumulative effect is observed on the lee side and atoll water flows out to the west whilst the relatively strong oceanic flow prevents outflow on the windward, east side.

The (almost) one-way water movement results in windward reefs being exposed to low-nutrient oceanic water for the entire period of each monsoon. Leeward reefs would be exposed to nutrient-rich (upwelled) water as tidal flow moves water in and out of the atoll. Reverse effects are observed during the SW monsoon. Currents are also moderated or increased by widths of channels (*Kandu* is the common *Dhivehi* term for channel and *kanduoli* is used for wide channels which do not funnel current). During the transition weeks between the monsoons, there is no overall flow direction and mantas are dispersed throughout the atolls, and were found on both sides and inside the atolls (Chapter 5).

During falling tides, the outflow to the lee side may pass over large submerged reefs on the edge of the atolls and it is common to observe mantas feeding at such places (Figure 3.6). Strong tidal currents in reef passages are known to induce upwelling of deep water from outside the reef by Bernoulli suction (Storz and Gischler 2011). It is proposed that strong outflow creates upwelling in the lee of the reefs, sucking water full of zooplankton and nutrients into shallower water. On the next tide (inflow) the enriched water is sucked into the atolls and on the following tide (outflow) it is sucked back out again. Mantas may be seen gathering over submerged reefs during outflows or where the reef traps outflowing plankton e.g. at Hanifaru or Guraidhoo Kandu. Manta feeding behaviour at the latter is reported in Chapter 8. In October and March, following a period of reduced wind and current conditions, a spawning event (fish, corals, sponges and other invertebrates) often occurs around the full moon (pers. obs.) and mantas may be seen feeding in very large numbers. This congregation of mantas is sometimes referred to as a “massing” and appeared to coincide with peak mating activity. The hypothesis that mating activity is related to food abundance is discussed in Chapters 8 and 10.

Earlier in this section it was noted that mantas were commonly seen at cleaning stations on the shallow reef close to outer reefs where the reef slope was shallow, creating a shelf or plateau before dropping off steeply. It is likely that the nutrient rich water full of phytoplankton and zooplankton (which had risen from the deep by upwelling) is sucked into and out of the atoll during tidal movements and is also contained in the near-shore area by the presence of the shallow shelf. These areas would therefore be likely to have higher concentrations of prey, which would be attractive to mantas. Anderson et al. (2011) suggested that as zooplankton enriched water was sucked into

the atoll during water movements, and that this was evidence of large quantities of plankton to be in the deep water on the downstream of atolls. They speculated that mantas often feed in the deep zooplankton at night. This was supported by reports of mantas travelling to deeper water at night by Homma et al. (1999), Dewar et al. (2008) and Clark (2008). Further investigations are needed to understand the mechanisms of how zooplankton and nutrient enriched water enters the atolls (as zooplankton surveys are lacking from the Maldives), but it is likely that the complicated combination of geography, winds, tides and nutrient distribution combine to create accessible food sources for mantas in shallow waters. Whilst in these shallow water mantas are likely to be attracted to nearby cleaning stations where they are observed and can be studied.

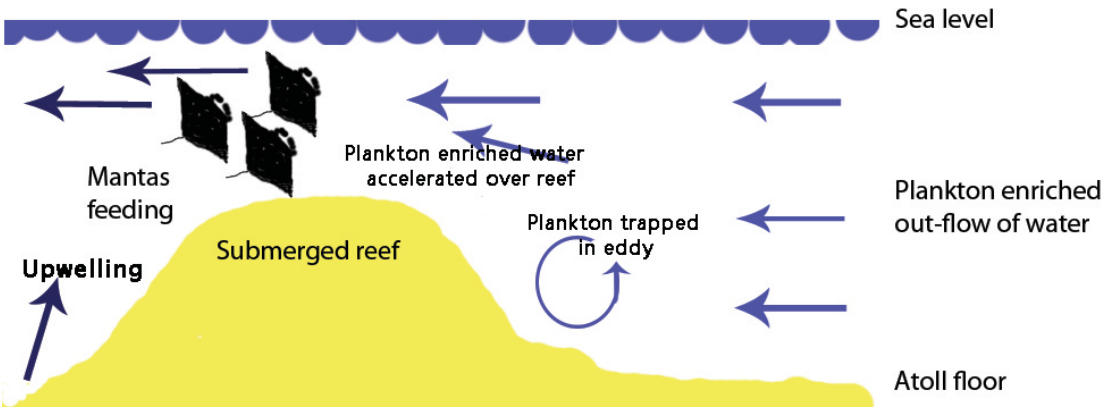


Figure 3.6 Diagram showing outflow of plankton enriched water eddying in front of, and flowing over submerged reef. The reef may concentrate the food, attracting mantas to feed in shallows above the submerged reef. The rapid outflow may also cause upwelling of nutrient enriched water and zooplankton on the downstream side of the reef.

3.5 Study sites

The Maldives atolls have two names, a geographical and an administrative one. The common usage name of the atoll may be either of these (Table 3.1). 27 sites (Table 3.2), distributed from Haa Alifu atoll in the north to Addu in the south (Figure 1.1) were studied. However, seven of these sites in two central atolls (North Male and Ari) account for 97% of all observations made; thus only these are described in detail (indicated in bold type in Table 3.2). The sites are principally cleaning stations but some are also feeding areas and have been marked with as asterisk in Table 3.2. The sites are all known “manta points” (as introduced in Chapter 1) and are commonly known by that term in each atoll e.g. “Maavaru manta point” in North Nilandhe etc. Each of the sites listed is a recognised diving point (dive site) and the name of the dive site is used throughout this thesis to differentiate “manta points”. The name may be a *Dhivehi* or

English name as both languages are in common use in the naming of sites (Harwood and Bryning 1998; Godfrey 2006). In some cases the name has geographical relevance, the site being named after a nearby island or reef (e.g. Helengeli Thila). In other cases they are just given names (Emas thila, Fairytale Reef etc.). English names (and sometimes Italian or Japanese names) have been adopted by local divemasters and boat crews and will be considered proper names in this study. When a *Dhivehi* word is used and the meaning is relevant, an English translation is given when the word is first used. On larger scale maps the coloured dots represent the locations where the mantas were commonly sighted and where surveys were conducted.

Administrative name	*Geographical name*
Haa Alifu	North Thiladhunmathee
Haa Dhaalu	South Thiladhunmathee
Shaviyani	North Miladhunmadulu
Noonu	South Miladhunmadulu
Lhaviyani	Faadhippolhu
Kaafu	**North Male**
Kaafu	**South Male**
Vaavu	**Felidhoo**
Raa	North Malhosmadulu
Baa	South Malhosmadulu
Alifu	**Ari**
Faafu	**North Nilandhe**
Meemu	**Mulaku**
Seenu	**Addu**

Table 3.1 List of the common names (bold) of atolls used throughout this thesis, together with the corresponding geographical or administrative name.

The probability of observing mantas varied considerably among sites, even during a single monsoon season (this is investigated in Chapters 5 and 7). Since the primary purpose of the surveys was to gather data on mantas, previous experience was used to schedule visits to survey sites so as to maximise the likelihood of encounters. However, for particular studies (being cleaned study (Chapter 7), population study (Chapter 6)), surveys were planned over prolonged periods of time (6 to 11 hours each day) to investigate variations in numbers of mantas observed. Full methods for these studies are described in the relevant Chapters. For each site the following information is given: 1)

location, including map reference, 2) a diagram of the site, 3) specific information about the survey site including precise locations of cleaner fish aggregations, small scale topography and currents.

Atoll	Survey site	Site letter	Latitude and longitude (Degrees and decimal minutes)
Haa Alifu	Desperation Thila	E	6º 46.396'N, 73º 05.067'E
Shaviyani	Koshibee *	J	6º 18.522'N, 72º 58.678'E
Noonu	Manadhoo	C	5º 45.758'N, 73º 25.139'E
Raa	Kottefaru & Beyrian Thilas	T	5º 41.865'N, 73º 00.633'E
Lhaviyani	Fushifaru Thila	Y	5º 29.665'N, 73º 31.398'E
Baa	Dhonfanu Thila	V	5º 17.178'N, 73º.11.606'E
	(includes Dhigu Thila and Hanifaru*)		5º 17.430'N, 73º 10.560'E
	Kunfunadhoo Thila (Nelivaru)	X	5º 12.508'N, 73º 07.562'E
North Male	Helengeli Thila	(N/A)	4º 63.613'N, 73º 56.880'E
	Fairytale Reef	**F**	4º 32.773'N, 73º 37.547'E
	Prisca	P	4º 48.963'N, 73º 68.904'E
	Boduhithi (2 adjacent sites)	**B**	4º 26.870'N, 73º 21.834'E
	Aquarium & Kani	N	4º 20.822'N, 73º 37.134'E
	Sunlight Thila	**S**	4º18.030'N, 73º 31.987'E
	Lankan Reef	**L**	4˚16.758'N, 73˚ 33.388'E
South Male	Guraidhoo* (Sandune)	G	3º 53.807'N, 73º 27.757'E
Ari	Rasdhoo North Channel	R	4º 19.423'N, 73º 00.399'E
	Ukulhas Thila	U	4º16.171'N, 72º 49.568'E
	Table Thila	**D**	3º 58.176'N, 72º 42.806'E
	Himendhoo & EmasThilas	H	3º 54.952'N, 72º 43.191'E
	Kalhahandi Huraa	**K**	3º 47.600'N, 72º 42.350'E
	Madivaru	**M**	3º 35.000'N, 72º 43.100'E
Felidhoo	Dhiggiri	I	3º 38.933'N, 73º 29.426'E
	Alimathaa	A	3º 35.488'N, 73º 30.156'E
North Nilandhe	Maavaru	W	3º 07.484'N, 72º 52.026'E
Mulaku	Kurali	0	2º 45.523'N, 73º 22.784'E
Addu	Mudakan	Q	0º 36.420'S, 73º 09.170'E

Table 3.2 List of main manta survey sites visited in this study (Figure 3.7). Sites marked with as asterisk are principally feeding sites but include at least one cleaning station. The names given are those in common use for the site. Each survey site is allocated a unique letter which is used to indicate the site at which each manta was first identified in the unique code assigned to each individual. When sites are very close (< 1 km apart) were treated as a single survey site. 97% of manta observations were made at the seven sites indicated in bold type.

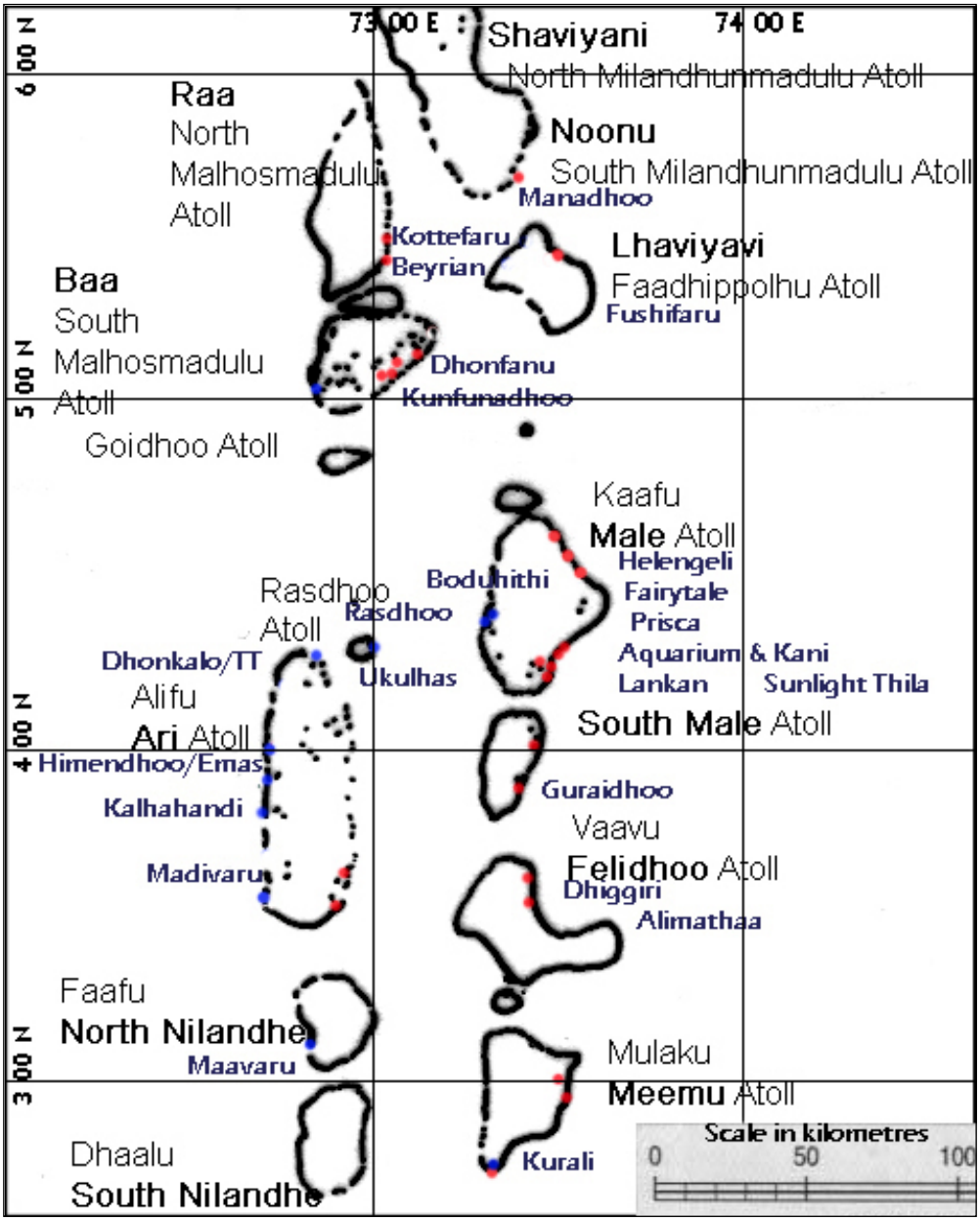


Figure 3.7 The locations of survey sites in the central atolls. Colour spots indicate in which monsoon season mantas are observed. Red indicates SW monsoon; blue indicates NE monsoon. Sites where mantas may be seen in both season have spots of both colours (Kurali). Haa Alifu and Addu atolls sites are omitted.

3.5.1. *North Male atoll*

Of at least 12 known "manta points" in North Male, eight are regularly visited by divers and included in this study (Figure 3.8, Table 3.2). In addition, there are several other sites where mantas are commonly seen feeding but very few manta identifications have been made (1 or 2 per site). Only the three main sites of Lankan Reef, Sunlight Thila and Boduhithi are described in detail.

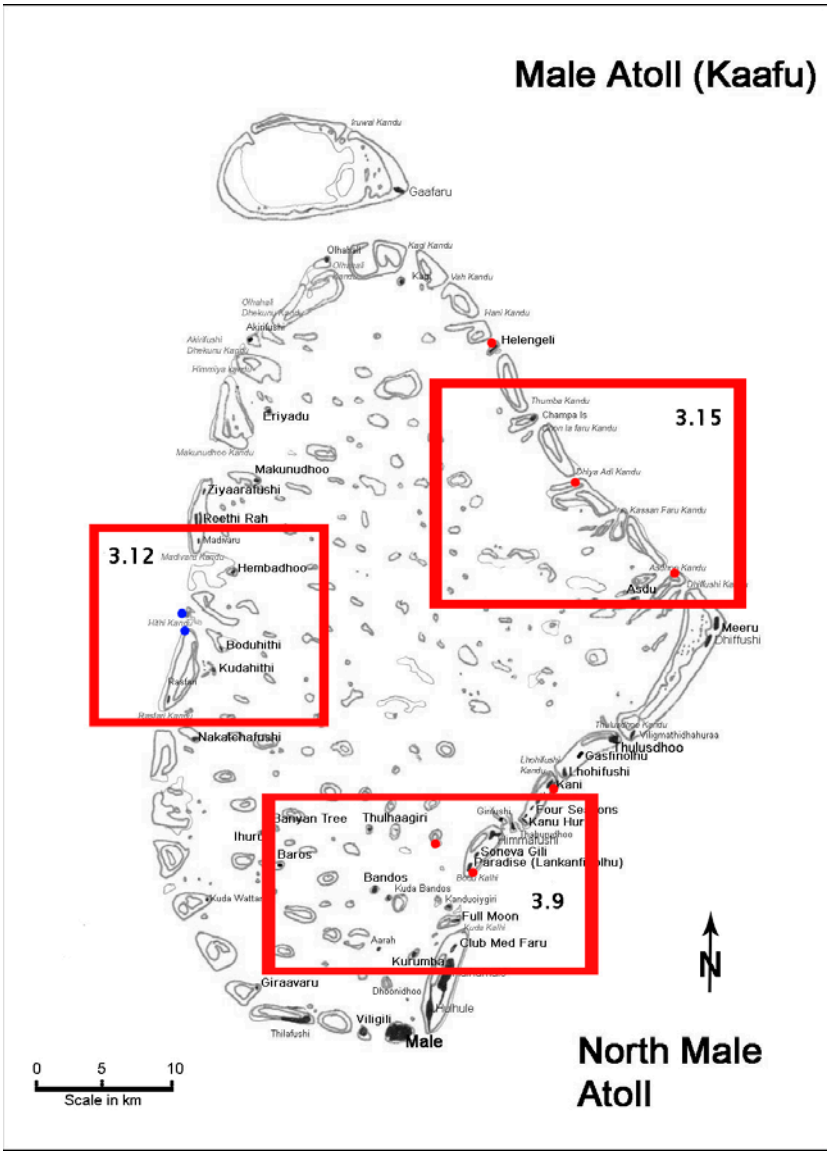


Figure 3.8 A diagram of North Male Atoll to show locations of 8 sites where manta surveys were carried out. Each is marked with a coloured spot. Red and blue dots indicate reef sites visited during the SW and NE monsoons respectively. Boxed areas are illustrated in detail in Figures 3.9 (bottom right), 3.12 (middle left) and 3.15 (top right).

Lankan Reef (also known as Lankanfinolhu Beyru, Manta Point, Paradise Reef)

To the southeast of Lankanfinolhu island (Paradise Island Resort- Figure 3.9) the shallow fore reef extends approximately 100 m beyond the beach at a depth of less than 10 m (Figure 3.10). The reef then drops off more steeply (10 to 35 m) and then continues to slope gently until several kilometres offshore where the atoll edge is reached and depths plummet to 2000 m. The site is the southernmost ocean facing reef before the deep channel separating Lankanfinolhu and Furanafushi islands. Observers are directed to visit Lankan reef during the SW monsoon (May to November) when it is in the lee of the prevailing winds and is normally sheltered even in the most extreme weather conditions (Harwood and Bryning 1998). Mantas have been sighted as early as April and as late as December in years with early or late monsoon changeovers. Adverse conditions including strong surge and currents make this site potentially dangerous (see appendix A for diving instructions). A single event affecting manta

behaviour was the building of Paradise Resort at Lankanfinolhu island from 1994-1995. Following dynamiting to clear the lagoon to the west of the island, mantas ceased visiting the cleaning station at Lankan Reef and were instead observed at Sunlight Thila (located inside the atoll about 2 km distance from Lankanfinolhu). By 2003 the mantas had started to return to Lankan Reef and within two years mantas were rarely seen at Sunlight. Sunlight continues to be visited as it is an easy, beginner's diving site.

Figure 3.9 Map to show detail from Figure 3.8 of south-east North Male atoll. The locations of Lankan reef and Sunlight thila are indicated by red spots. Lankan reef is on the eastern rim of the atoll and ocean facing whereas Sunlight thila is located near a faro just inside the atoll.

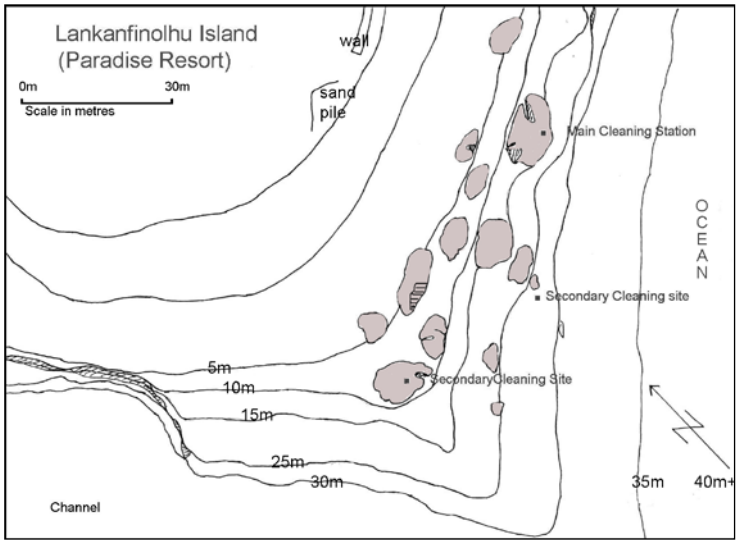


Figure 3.10 Diagram of Lankan Reef to show the main (4˚16.758'N, 073˚33.388'E) and secondary cleaning stations along the deep fore reef. The Main Cleaning Station is where the majority (>90%) of mantas were observed but they can be seen all along this reef. The section of reef shown in this diagram is approximately 150 m east to west. Contour lines indicate depth of water in metres. The grey patches indicate large *Porites* bommies.

The main manta activity is found amongst large, mainly dead, *Porites* coral heads (4-10 m in diameter); which lie along the drop off for the entire length of the reef (Figure 3.10). These overhang the reef slope at 8-22 m depth. The *Porites* heads have other coral species living on them and have resident reef fish including cleaner species. Nearly all the blocks have colonies of cleaner fish and are cleaning stations for many fish. Mantas are mainly seen being-cleaned around the block indicated as "main cleaning station" in Figure 3.10, but they can be seen all along this outer reef including the section of reef 2-300 m north of the section shown in Figure 3.10. A study of the cleaner fish and mantas being-cleaned at this site is reported in Chapter 7.

Sunlight Thila

Sunlight Thila is a shallow reef rising to 4 m below the surface, located approximately 80 m to the south-east of Kubuladi Faro, inside the south-east part of North Male atoll (Figure 3.11). *Thila* is the *Dhivehi* word meaning submerged reef, normally defined as one deeper than 3-4 m that a local boat can safely navigate over (a shallower reef, just below or just breaking the surface would be a *giri* or *faru*).

The survey site consists of a number of coral blocks scattered across the sand floor between Kubuladi Faro and Sunlight Thila. The blocks vary in size from approximately 1 m in height and diameter to 2.5 m height and 4 m diameter and are of various coral species. Healthy *Porites* grow on some blocks and there are some large *Acropora* Table corals (> 2 m diameter) near the deep cleaning station. Most of the coral blocks have colonies of cleaner fish in them. The species and numbers of each seen are reported in Chapter 7. Mantas may be seen at any of the blocks but were mainly seen at the "Deep" and "Shallow" cleaning stations (Figure 3.11).

This site was the main manta observation site for dive tourists in the south part of North Male atoll from 1995-2003 owing to construction disturbances at Lankan reef. By spring 2004 mantas were mainly reported at Lankan Reef and observations at Sunlight Thila had markedly decreased. The site is only accessible by boat as it is distant from any resort and entry is usually made directly onto the "Deep cleaning station" using the GPS mark.

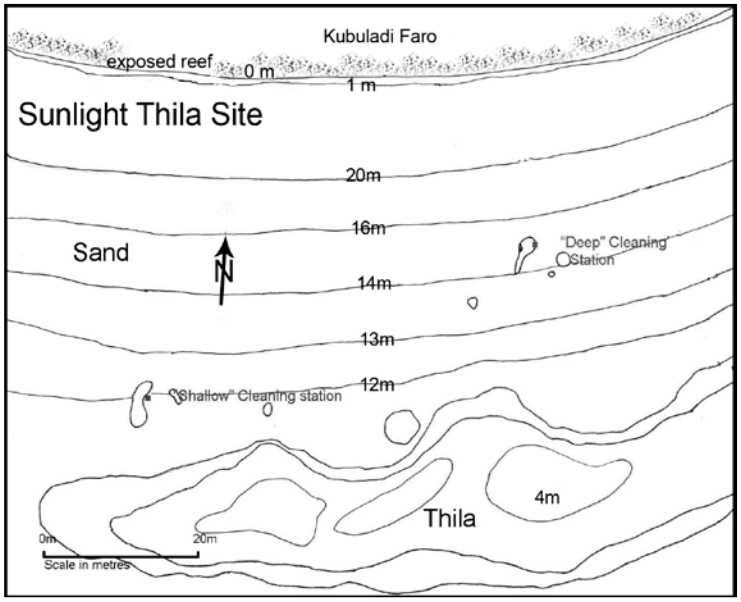


Figure 3.11 The location of Sunlight Thila with respect to Kubuladi Faro. The two cleaning stations where surveys were carried out are located on the sand slope between the faro and *thila* and not on the *thila* itself. Contour lines indicate depth of water in metres. The GPS location for "Deep Cleaning Station" is 04º 18.030'N, 073º 31.987'E.

Boduhithi (Rasfari North and Boduhithi Thila)

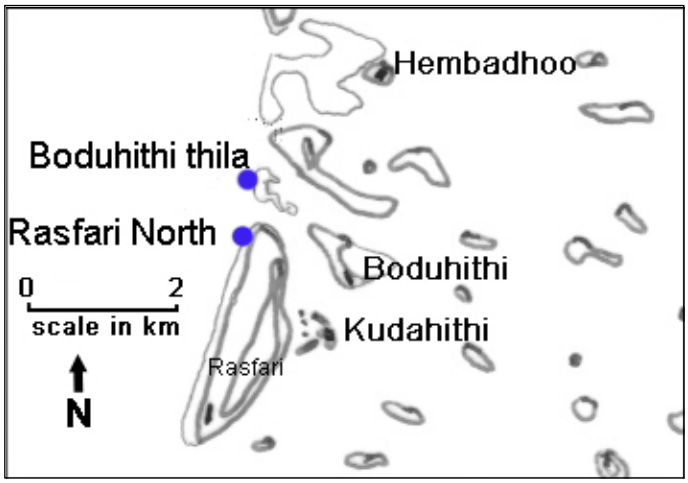


Figure 3.12 Detail from Figure 3.8 to show the location of Boduhithi Thila and Rasfari North sites on the western rim of North Male atoll. The sites are on opposite sides of a 40 m deep channel. The GPS coordinates for Rasfari North and Boduhithi Thila are 4º 26.500 N, 73º 21.700'E and 4º 26.870'N, 73º 21.834'E respectively.

These two reefs are located approximately 500 m apart and are treated as a single survey location as a manta may swim from one to the other within minutes. Rasfari North is on the north-west of the fringing reef surrounding Rasfari Island lagoon (Figure 3.13). Boduhithi Thila is a large (500 m x 500 m) submerged reef rising to a flat top at 8 m located in the Hithi channel directly north of Rasfari Island lagoon. Both sites are regularly visited by divers to observe mantas. Rasfari North was one of the three sites chosen for diel observations (see Chapter 7). The two sub-sites are markedly different and will be described separately.

Rasfari North

Divers and snorkelers are directed to visit this site to observe mantas during the NE monsoon period, particularly mid-December to mid-March, when this reef is in the lee

of the prevailing winds and is normally sheltered even in the most extreme weather conditions (Harwood and Bryning 1998; Godfrey 2006). This site is unusual because it is so shallow, with most observations in <2.5 m. The entire north-east section of the outer reef consists of spur and groove starting at the reef crest at 6 m, and continuing for more than 50 m becoming progressively shallower (< 1 m depth): the reef flat harbours large populations of juvenile wrasse (Labridae), parrotfish (Scaridae) and snapper (Lutjanidae). Distributed across the reef are small *Acropora* and *Pocillopora* colonies of maximum diameter 50 cm, but there is low coral cover due to wave action during the SW monsoon when this reef is subject to significant swell.

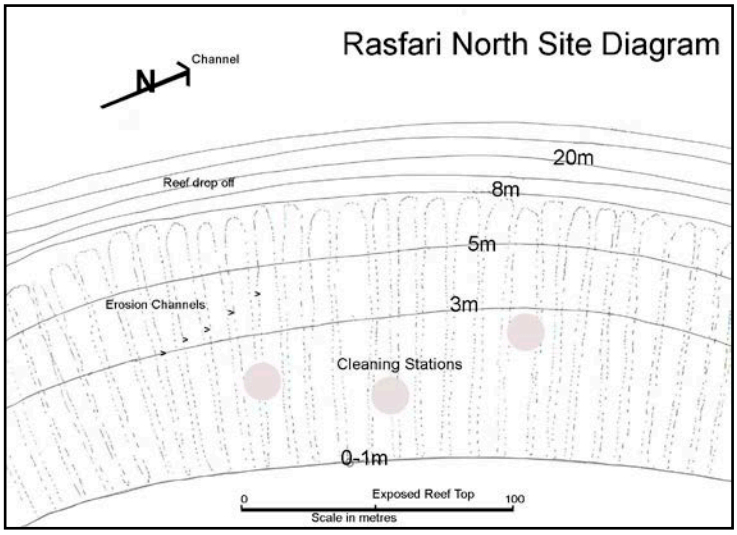


Figure 3.13 Diagram of Rasfari North site showing detail of the section of reef where mantas are most commonly seen. The preferred cleaning stations (where mantas are most frequently reported) are indicated in pale grey and harbour concentrations of the cleaner fish. Contour lines indicate depths in metres.

The cleaning stations visited by mantas are colonies cleaner fish located in a specific area of reef flat, approximately 100 m x 25 m. This is shown diagrammatically in the centre of Figure 3.14. The mantas visit cleaning stations at 2-3 m depth and no deeper, even though large numbers of cleaner wrasse are distributed over the reef to 8 m+ depth and further north and south of this area. Mantas can easily be spotted from boats by their wingtips and dorsal fins breaking the surface, the dark dorsal surface of a manta contrasting strongly with the pale reef so an entry into the water 20 m from where the mantas are sighted is usually made.

Boduhithi Thila

Boduhithi Thila is a large rectangular submerged reef located in the Hithi Channel to the north of Rasfari Island and to the north-west of Boduhithi Island and their fringing reefs. The *thila* is in a channel and is subject to strong tidal current flow. To the eastern margin of the *thila* is an area of sand to a depth of 20-30 m which extends to another

submerged reef system further east. To the north and south of the *thila* are channels of ~40 m maximum depth. The western margin faces deep water.

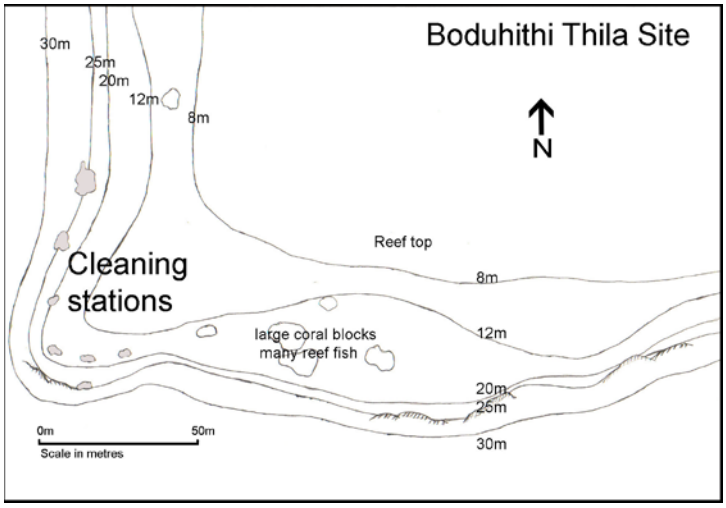


Figure 3.14 Diagram showing the area of Boduhithi Thila where mantas may be observed. Contour lines indicate the depth in metres. The main observation area is located on the south-west corner at the coral blocks shaded in pale grey and on the reef slope in the area marked "Cleaning stations".

This *thila* is a relatively large site and mantas have been sighted in the area throughout the NE monsoon (from December to late April). Different mantas have been simultaneously observed being cleaned on the reef and feeding in the shallows where plankton is siphoned over the top of the *thila* in suitable current conditions. Although mantas can be sighted anywhere on the *thila* (being-cleaned or feeding), the south-west corner (Figure 3.14) is the most reliable site to observe cleaning activity (pers. obs.). The cleaning stations are either coral blocks approximately 1.5 m in diameter located at 14-25 m depth on the SW reef slope, or at colonies of cleaner fish on the reef crest.

Fairytale Reef

Fairytale reef is located on the north-east of North Male atoll (Figure 3.15). The main reef is on the south side of the channel known at Dhiya Adi Kandu and is the northern fringing reef of a large lagoon. The cleaning stations where mantas are most likely to be observed are located at large coral blocks in the north-eastern part of the lagoon and occasionally at coral blocks on the top of the main reef parallel to the blocks. The GPS mark for the main coral block is: 04º 32.773'N, 073º 37.547'E. Mantas were typically only active at this site from August to November.

The horseshoe shaped lagoon on the south side of Dhiya Adi Kandu acts as a natural trap for plankton. Plankton washed into the atoll on a rising tide may be trapped in the lagoon by the shallow reefs on the north, east and south of the lagoon (see Figure 3.15 for shape of lagoon, the tips of the horseshoe lie to the west). Mantas were frequently

observed feeding on the surface within the lagoon as well as visiting the cleaning stations located at the east of the lagoon (Figure 3.16). Surveys were only conducted on mantas visiting cleaning stations.

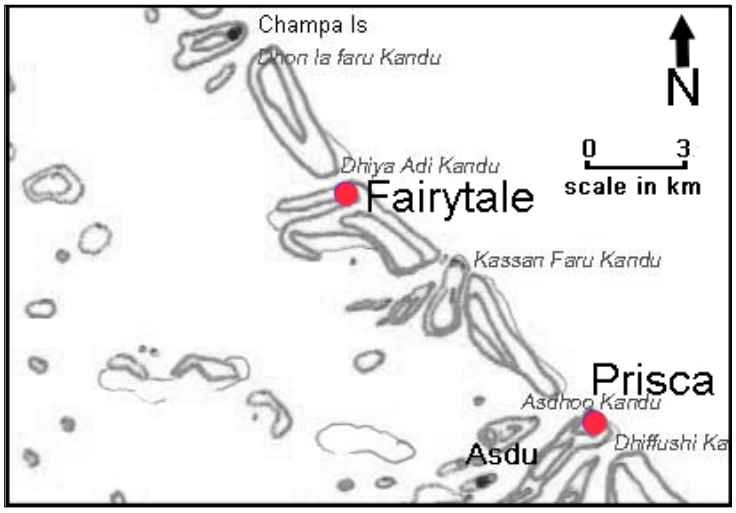


Figure 3.15 This map shows detail from Figure 3.8 with Fairytale Reef located at the north-east of the fringing reef bordering Dhiya Adi Kandu. The nearby "manta point" called Prisca is also shown.

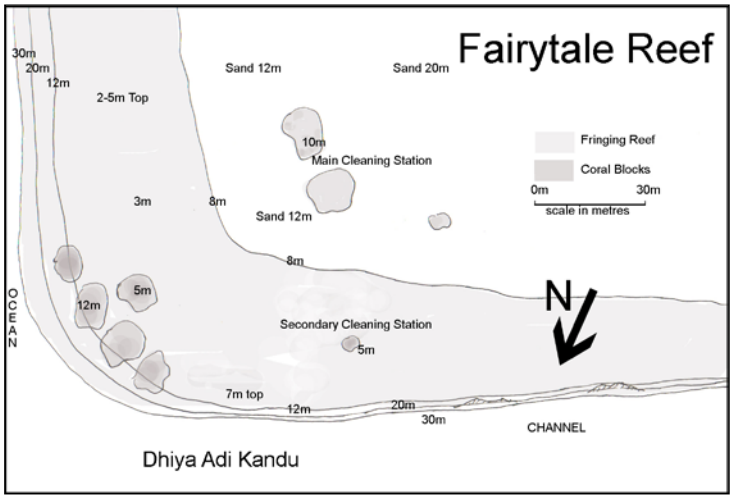


Figure 3.16 Map of Fairytale Reef showing "Main Cleaning Station" in the sand-bottomed lagoon inside the north-east fringing reef on the south side of Dhiya Adi Kandu. Contour lines show depth in metres. Note the east facing side is much shallower (rising to 2 m depth). The section of reef shown is approximately 200 m east to west.

3.5.2 *Ari Atoll*

Ari is split into North and South administrative regions (Figures 3.17 and 3.18). North Ari includes the small geographical atolls of Rasdhoo and Thoddhoo to the north-east of the main atoll. The atoll is famous for its manta sightings during the NE monsoon (Harwood and Bryning 1998; Godfrey 2006) but mantas were not regularly sighted in the SW monsoon even though the eastern side of the atoll appears to offer appropriate sheltered sites. Mantas were occasionally sighted at Table Thila (Figure 3.17) in North

Ari atoll during the SW monsoon during periods of low winds and tides but these sightings account for less than 1% of total sightings.

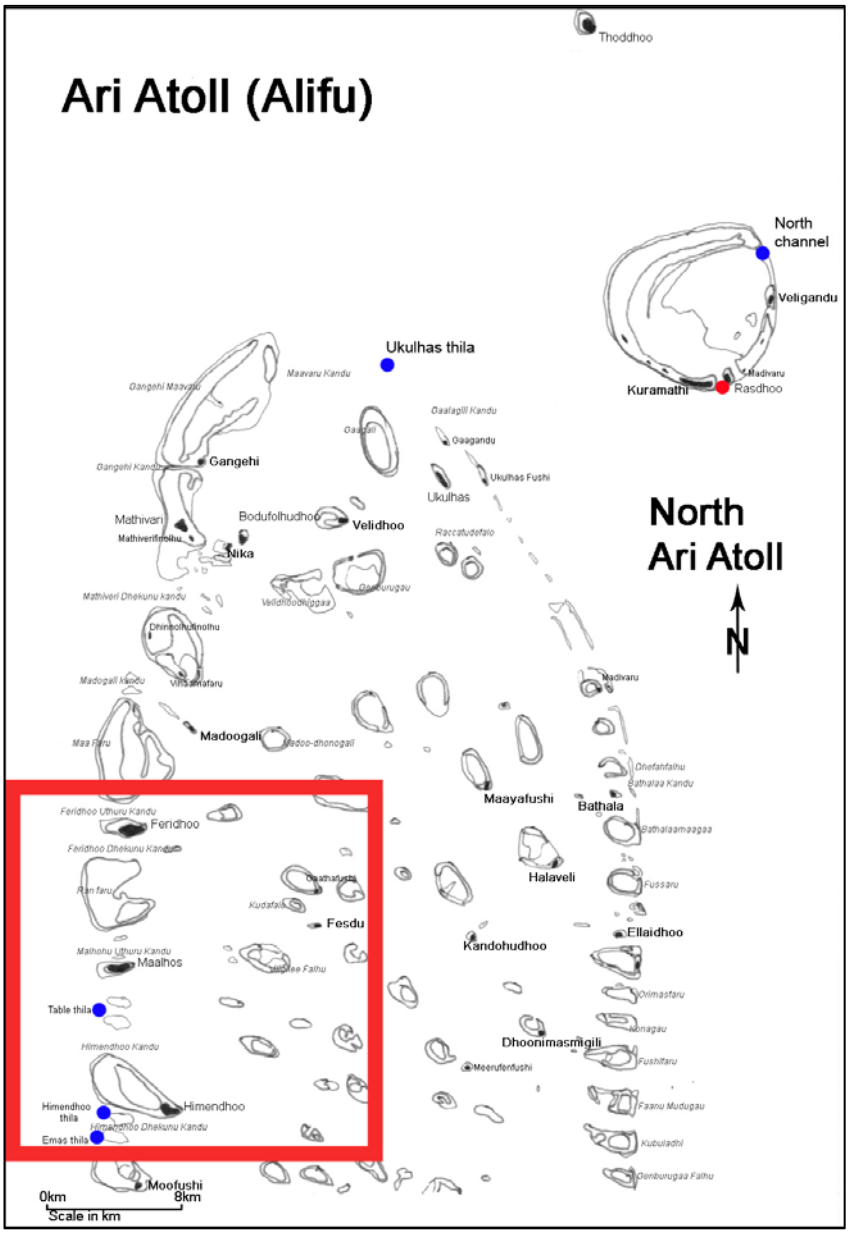


Figure 3.17 Map of North Ari atoll with area around Table Thila, Emas Thila and Himendhoo Thila outlined in red (see Figure 3.20 for detail). Rasdhoo and Thoddhoo atolls are located to the north-east. NE monsoon sites where mantas were seen are marked with blue spots and SW monsoon sites are marked in red.

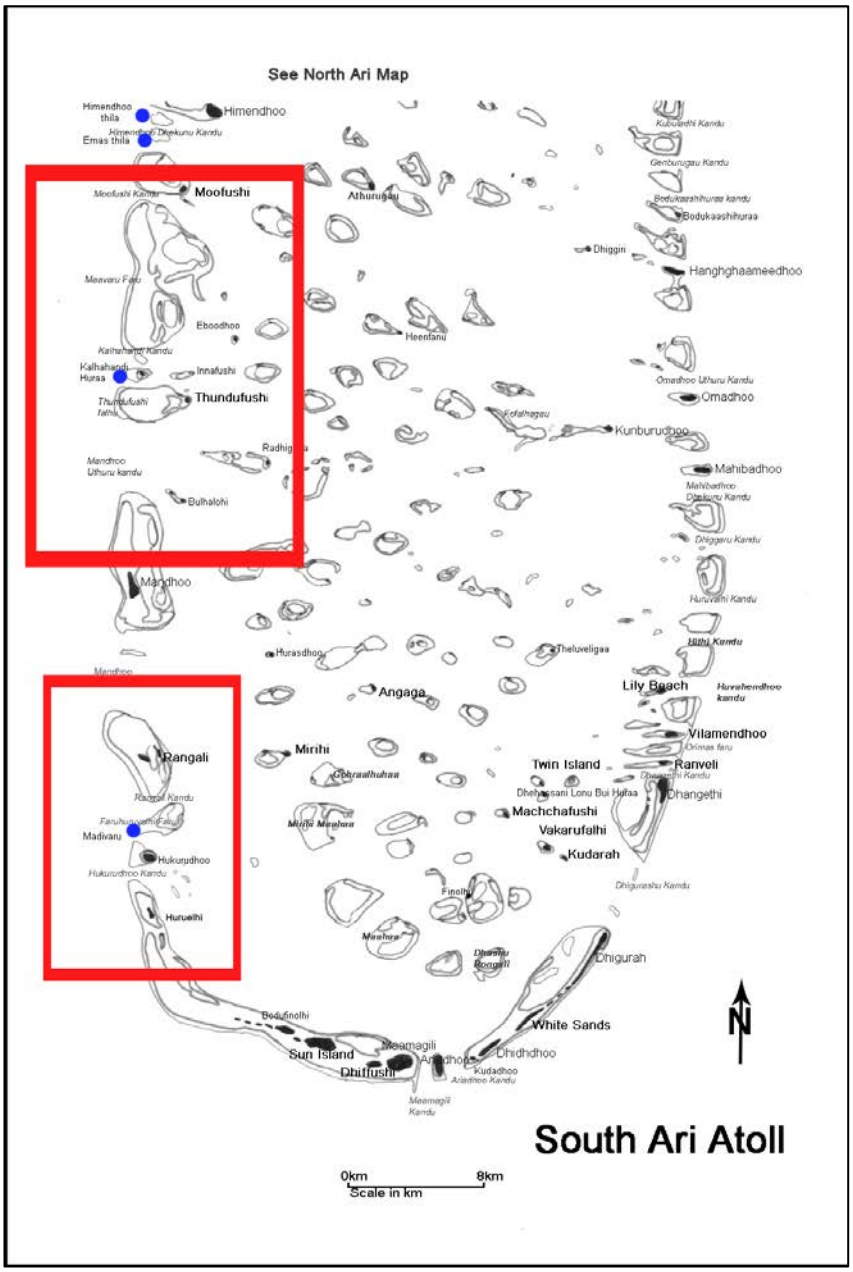


Figure 3.18 Map of South Ari atoll to show locations of Kalhahandi Huraa (upper left section outlined in red, and detailed in Figure 3.19) and Madivaru (lower left section outlined in red, and detailed in Figure 3.22). NE monsoon sites where mantas were seen are marked in blue. There are no SW monsoon sites where mantas are reliably seen in this area.

Table Thila

The survey site known as Table Thila is located at the western margin of a submerged reef in the channel (Himendhoo Kandu) between Maalhos and Himendhoo islands (Figure 3.19); and is an excellent site to observe mantas during the NE monsoon, particularly from late December until early April (pers. obs.). The site was only discovered in late 2002 and was not a known dive-site or "manta point" prior to that time although mantas were reported in the channel (Godfrey 2006). Mantas have also been occasionally sighted here during the SW monsoon. Table Thila is the southerly of two submerged reefs separated by a shallow channel with a sand and coral-limestone rock bottom. The northern reef is known as Dhonkalo Thila and was an established dive site prior to 2002 (Godfrey 2006). The southern reef had no local name but is known to

the author as Table Thila to differentiate it from the northern reef. This site is referred to as Table Thila henceforth.

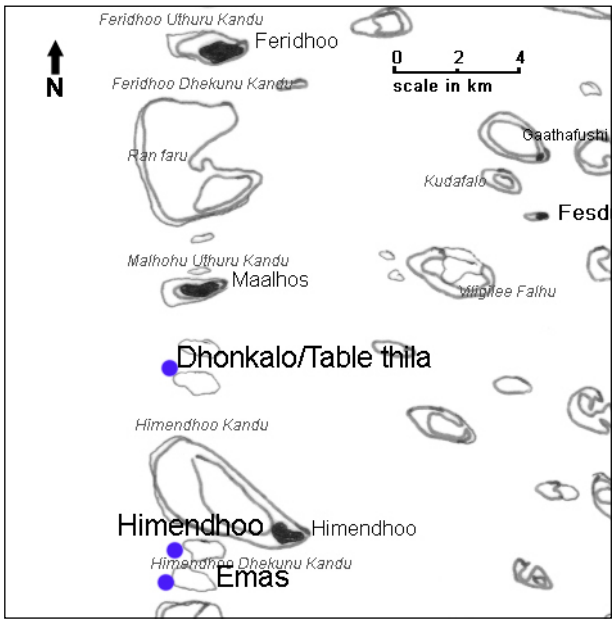


Figure 3.19 Detail from Figure 3.18 to show location of Dhonkalo Thila and Table Thila These sites are located south of Maalhos island and north of Himendhoo island in Himendhoo Kandu. There are at least two submerged reefs in the channel, the northern one is Dhonkalo Thila and the southern one is Table Thila. The blue dot indicates the area where mantas are seen. In the channel south of Himendhoo island the areas at Himendhoo Thila and Emas Thila where mantas are seen are also marked.

Table Thila is approximately 350 m from east to west and pear shaped. The narrow end faces west to the deep ocean. To the east, the reef drops off steeply to 35 m and eventually 50 m within the atoll. The north faces Dhonkalo Thila, from which it is separated by a sandy channel. To the west, the thila slopes off gently to a sand and limestone base (Figure 3.20). Depths of 60 m can be found approximately 100 m west of the thila and further west the drop off to 2000 m occurs. There appears to be a shelf at about 60 m depth along the western side of Ari atoll as far north as Madoogali Kandu, located a little north of the area marked with the red box in Figure 3.18 (Purdy and Bertram 1989). The GPS mark for the site at the western end of the sand channel is 03º 58.176'N, 072º 42.806'E.

The cleaning stations are located on the north and north-west margins of Table Thila. The smaller cleaning station area is located close to where the sand ridge peaks, but on the *thila* itself and is only identifiable from the concentration of cleaner wrasse there. The area (c.3x3 m) is indicated by the smaller dark grey-shaded circle to the south of the sand ridge in Figure 3.20. The other main cleaning station area (c.150 m²) is the reef slope and the coral-limestone at the north-west margin of the reef indicated by the larger dark-grey shaded area to the left of Figure 3.20. Sometimes the mantas are cleaned on

the top of the *thila* or at the western deep reef slope (30-35 m depth) as cleaner fish are located all over the site.

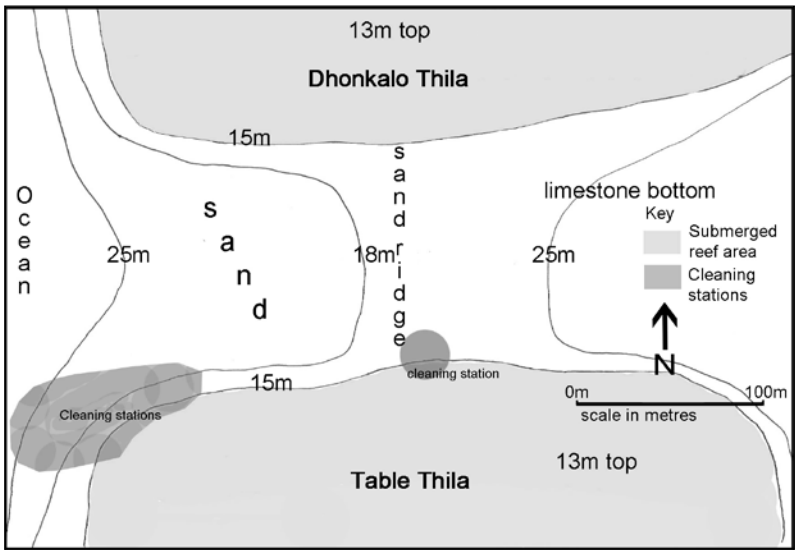


Figure 3.20 Diagram to show the two submerged reefs of Dhonkalo and Table thilas and the shallow channel between the reefs with a sand base to the west and rising to a ridge at approximately 18m depth. The two most common cleaning station areas are indicated.

The *thila* has dense coral growth on the top at 13 m extending to the base, which varies in depth, but is at approximately 20 m from the centre to the western extreme of the thila. The base is a flattened area of limestone rock which sometimes becomes smothered by the drifting sand. There are small areas of coral growth on the base and in particular are some 80 cm diameter red/black sea fans at the western end where mantas are often seen and these can be used to identify the correct area of reef for surveying divers to wait by if mantas are not already present. There are various fish species all over the reef and juveniles are particularly evident.

Kalhahandi Huraa (also known as Pannetone)

Kalhahandi Huraa is the name of a sandbank in South Ari (see Figure 3.19). *Huraa* is the *Dhivehi* name for a sandbank with high piled sand but no vegetation. The sandbank is located on the central western margin of Ari atoll in the Kalhahandi Kandu, north of Thundufushi Island and *falhu* (Figure 3.21). The entire northern reef of the sandbank is a famous dive site but the area where mantas are usually seen (and was the survey site) is located at the far W/NW reef margin (Figure 3.22).

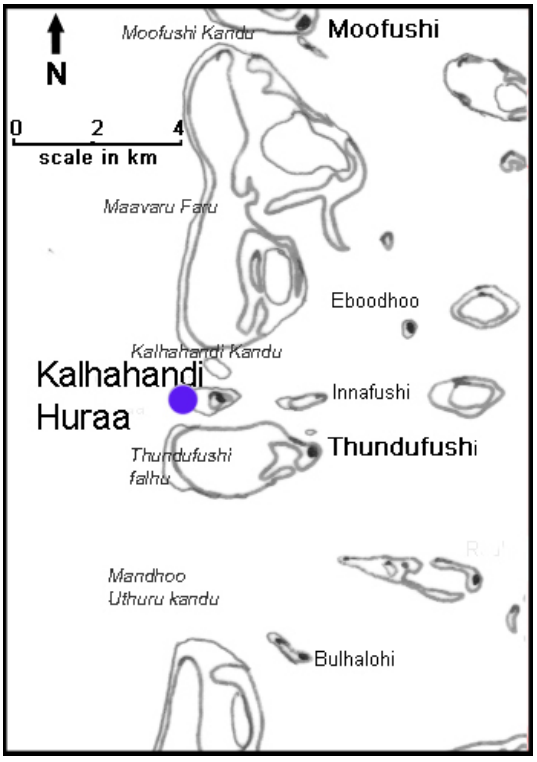


Figure 3.21 Detail from Figure 3.18 of area surrounding Kalhahandi Huraa. The area where mantas are typically seen is marked with the blue dot. The actual sandbank (*huraa*) lies to the east of the cleaning station.

The sandbank reef system consists of an extensive fringing reef on all sides except the south which is mainly sand slope (Figure 3.21). Kalhahandi Kandu is the channel to the north of the sandbank and is approximately 40 m deep with a large submerged reef (Kalhahandi Thila) located centrally between the sandbank and the main fringing reef to the north (Maavaru Faru). Mantas were occasionally seen being cleaned at cleaning stations on the thila or feeding on top of the thila but no identifications were made so no data were included in this study.

The cleaning stations visited by mantas were located at the extreme western margin of the reef which extends approximately 500 m west from the sandbank (Figures 3.21 and 3.22). Cleaning stations were located almost continuously along the western margin of the reef, but mantas were most likely to be seen in areas sheltered from the current. The location of mantas varied with current strength and direction. Mantas were not reported at the cleaning stations during periods of current in-flow. Currents to 2 $m.s^{-1}$ have been experienced in this channel. Throughout the NE monsoon dense plankton is found around this site attracting mantas and even whale sharks *Rhincodon typus.* Underwater visibility was usually low (<10 m horizontally). Coral growth was exceptional. The north plateau was entirely covered with large *Acropora* Table corals up to 3 m in diameter which had all grown since the 1998 ENSO bleaching and mass mortality event. The ocean facing western reef is exposed to strong wave and surge action

throughout the SW monsoon and coral growth was not as prolific. There is extensive spur and groove development on the plateau with the depth of some of the grooves exceeding 1.5 m.

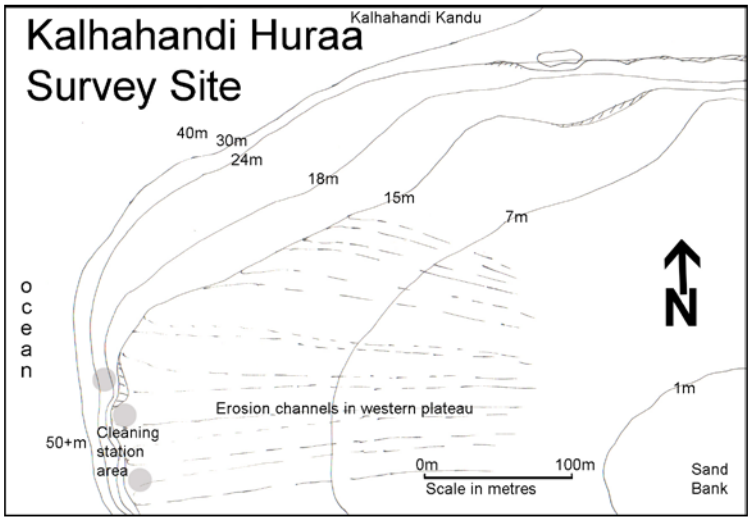


Figure 3.22 Detail of the western margin of the fringing reef of Kalhahandi Huraa to show the cleaning stations (marked by grey circles) located on the top and the edge of the western plateau drop off. The contour lines indicate the depth in metres. The GPS mark for this area at Kalhahandi Huraa is 3º 47.600' N, 72º 42.350' E.

Madivaru

Madivaru is the common name for the western, ocean facing section of Faruhuruvalhi Faru in south-west Ari atoll (the lower area indicated in red in Figure 3.18 and detailed in Figure 3.23). *Madivaru* literally means "manta reef" in *Dhivehi* and this site has been known by this name throughout living memory by fishermen who observed congregations of feeding mantas around this reef as they passed in and out of the atoll to fishing sites. In the channel between Faruhuruvalhi and Hukurudhoo Island large numbers of mantas are still regularly observed feeding over the submerged reef structures. The western plateau of Faruhuruvalhi extends approximately 200 m west from the shallow reef, and it is the north side of this western extension that is known as Madivaru dive site (Harwood and Bryning 1998; Godfrey 2006). Cleaning stations, which are visited by a multitude of fish species, are located all along the north-western and western margin of the reef. Mantas have been more common at the more western cleaning stations in recent years.

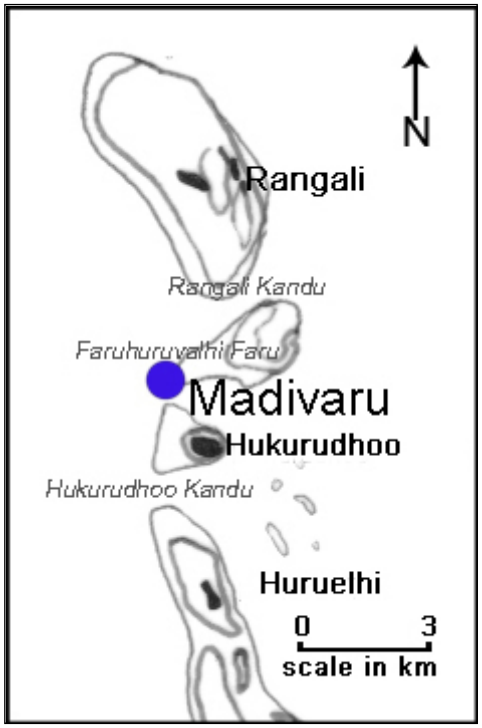


Figure 3.23 Detail from Figure 3.18 to show Madivaru site on the western tip of Faruhuruvalhi Faru. The GPS mark for Madivaru is 3º 35.000' N, 72º 43.100' E.

From December to early April (NE monsoon), mantas are regularly observed around Faruhuruvalhi reef, either feeding on the surface in the channel to the south or along the reef edge. Mantas are occasionally seen at this site during the SW monsoon during periods of calm weather and small currents. Mantas are normally observed during current outflows which tend to occur on a falling tide although they may be seen during very slow (less than 0.2 $m.s^{-1}$) inflows. There is a continuous fringing reef south of Hukurudhoo Island (Figure 3.18) starting at Huruelhi (west) and ending at Maamagili (south-east). Water can only flow in and out of the atoll on the west side in the channels north of Huruelhi. As discussed in section 3.4, strong out currents (caused by funnelling in the SW channels) are likely to cause upwellings of nutrients and plankton which are then sucked into the channels on the next tide change. The long C-shaped reef is likely to act as a barrier to the outflow of water/plankton, causing water flow to be directed out of the channels between Huruelhi, Hukurudhoo, Faruhuruvalhi and Rangali reefs. The mouth of the channel (Hukurudhoo Kandu) north of Hurueli is very shallow and this might create a barrier to flow causing more flow to occur in the deeper Rangali Kandu. With so much water funnelled through this channel the conditions required to cause Bernoulli suction, as alluded to by Anderson et al. (2011) become more likely. There is a concentration of manta and whale shark activity in this area (Riley et al. 2010) (pers. obs.), it is likely these zooplankton feeders are attracted by especially high food levels.

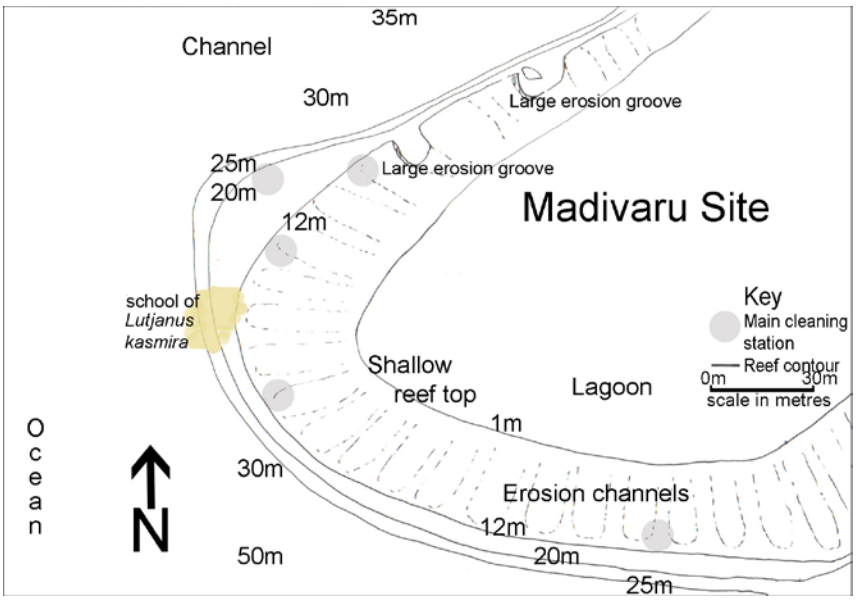


Figure 3.24 Diagram of Madivaru site which is the western section of Faruhuruvalhi reef. Grey shaded circles indicate main cleaning stations along the reef margins, however mantas can be observed cleaning anywhere along the north-western and western reef margin. Contour lines indicate depth in metres.

The reef consists of a flat plateau with a crest around 12 m depth. Within the channel the crest rises to about 7 m. The plateau slopes very gently and is heavily grooved by wave action creating spur and groove structures between 0.7 and 2.0 m in height and the grooves are tens of metres in length. The taller spurs are within the channel and are further eroded by eddying currents creating distinct semi-circular indentations at the reef crest (see Figure 3.24 for location). There are a number of large, indented erosion grooves within the channel but the two identified in the site diagram (Figure 3.24) are the most important as these were two primary manta cleaning stations from before 2000 until around 2003. The ocean facing reef slopes to a plateau at about 50 m (Purdy and Bertram 1989). The reef top has sparse coral colonisation with mainly *Acropora*, *Pocillopora* and *Porites* colonies.

Manta activity at this site seems to have decreased over recent years, probably due to diver pressure as it attracts tourist divers from the many resorts around South Ari atoll. This was tested by looking for a trend from the regression of mean number of mantas seen per survey each year between 2002 and 2009. A significant negative trend was found ($F = 4.6$, $P = 0.035$), supporting the hypothesis that the number of mantas sighted at Madivaru is decreasing. In March 2007 a full-day survey was carried out (for the population study reported in Chapter 6) and 15 other boats and over 120 divers were

counted visiting the site during a single day. The continual presence of divers is likely to deter mantas from visiting the site. The cleaning stations visited by mantas changed during the survey period. From 1997 to about 2003 it was common to see mantas along the north of the reef and particularly at the two areas identified on the site diagram (Figure 3.24) as “large erosion groove”. These two sites are well inside the channel and the large grooves are visually distinctive. Although these areas were still populated by cleaner fish during more recent surveys, mantas did not visit them regularly to be cleaned after 2004. The reason is unknown. Since 2004, most sightings were on the western margin. The north-outside corner is marked by a permanent school of blue-stripe snapper *Lutjanus kasmira* (Forsskål, 1775) at 15-30 m which create an ideal marker for divers visiting the site. There are many colonies of cleaner fish along the ocean facing reef. The colonies are not fixed, so cleaning station locations vary from one visit to the next depending on the location of the cleaner colonies. The two most reliable areas to see mantas on the ocean facing section are indicated in Figure 3.25 as grey circles, the first is a little south of the blue-stripe snapper school and the other is at the southernmost section of the ocean facing reef before the reef turns east into the south channel of Faruhuruvalhi Faru. Both areas are on the reef crest at about 12 m depth. There is a distinct spur on the inside of the channel where the reef slopes more gently. There is a cleaning station at about 20 m that was regularly occupied by mantas on an out-flow current.

3.6 Survey strategies and methods

3.6.1 *Observation strategies at the sites*

The majority of surveys were undertaken by the author as a secondary activity whilst guiding tourist divers. Additionally, there were 90 surveys during which the author was assisted by volunteer observers and the surveys were organised as research dives. The same observation methods were used whether on a specific research dive or as part of a tourist dive. Each dive/survey was typically of one hour duration. On all dives, a pre-prepared slate (Figure 3.25) and an underwater video camera were carried in order to record the details of any manta encountered. The primary objective of each dive was to identify which individual mantas were present using the identification method in Chapter 4. Secondary observations were also made on a range of behaviours.

Five main types of survey were undertaken at manta cleaning stations. These involved 1) identification of individual mantas, 2) recording the time spent by these mantas at the survey sites, 3) surveys of the abundance and species composition of the cleaner fish, 4)

surveys of the incidence of pregnancy in female mantas, and 5) observations of a range of behaviours (mating, feeding, agonistic interactions etc.).

i. Identification of individual mantas

Each animal present was photographed and recorded along with details of date, time and survey site (see Chapter 4 for full method). Survey periods were 15-90 minutes, with the majority (estimate >90%) in the 45-60 minutes range. A number of continuous 6-11 h observations, with observers working in rotation at the cleaning station, were also carried out, in order to survey diel use of a cleaning station and find out how many mantas visited a cleaning station during a day (see Chapter 7).

ii. Time spent at cleaning station

Times of arrival and departure were recorded for each individual manta identified. Mantas were continually coming and going over the cleaning station. Observations were reported in 5 minute periods. If a manta was absent from the observation area for more than 5 min, they were deemed to have departed. For example, if a manta arrived at the cleaning station at 09:02 was seen to swim away at 09:12, return at 09:15 and continue to be present until 09:30 before departing again, it would be recorded as being present from 09:00 to 09:30. A manta which arrived at 09:02, departed at 09:12 and was absent until 09:18 and then remained until 09:30 would be deemed present for two sessions from 09:00 to 09:10 and 09:20 to 09:30. Low horizontal visibility meant that a manta further than 20 m from an observer could not be seen, so it was difficult to tell whether a manta swimming away from the observer was leaving the vicinity of the cleaning station or just making a short swim for respiration purposes (see Chapter 7). Although this recording method was not precise, it was very difficult to identify the exact arrival and departure time of every animal unless only one or two animals were present. By rounding up and down within each five minute slot, most errors would be accounted for.

iii. Surveys of cleaner fish

Counts and species of cleaners at the site were recorded (methods for counting are reported in Chapter 7). For some surveys, underwater slates were pre-prepared with lists of species likely to be encountered so only a count of each needed to be recorded.

iv. Pregnancy in females

A note of observably pregnant females was made (see Chapter 10 for full method). The state of pregnancy was not recorded routinely in early surveys (2001 to 2004) although ad hoc records were made during this period. All females observed after June 2005 were checked to determine whether visibly pregnant and records maintained. Mantas are only visibly pregnant from around month 8 of gestation (see section 10.2) so that the majority of pregnant mantas would be missed during any survey.

v. Specific behaviour surveys

Specific behaviours (being cleaned (Chapter 7), feeding (Chapter 8), social behaviours (Chapter 9), and reproductive behaviours (Chapter 10)) were observed and recorded; captured using photographs and video and the sequences reviewed and analysed. Other than being cleaned, which was the main activity anticipated when observing mantas, all other behaviours were surveyed when opportunities arose. Video footage was recorded on Sony PC100, PC330 and HVR A1 digital video cameras. Photographs were primarily recorded using Nikonos V (film) and Canon D300 (digital) still cameras.

3.6.2 *Sampling strategy and issues*

The aim of all surveys was to find mantas to identify. Most surveys were planned at times when manta activity would be expected based on previous knowledge of the sites and tide state. Different sites required different conditions. 6-11 hour continuous surveys were carried out at Lankan Reef, Boduhithi, Table Thila and Madivaru so that the effects of tide and time of day could be studied and also at Hanifaru for the population study (Chapter 6). Mantas were observed at cleaning stations during the day from before 07:00h until 17:30h. There were few nocturnal surveys of feeding activity, one is reported in Chapter 8.

The number and frequency of sampling varied annually depending on the period of time spent by the author in the Maldives. Colleagues collected data during periods when the author was absent, but there were no years when data was continuous due to holidays and period of bad weather. The number of surveys conducted was not proportional to the time spent in the Maldives. For example, approximately 25% of all surveys were conducted during the 2006-2007 season (1 year) as the surveys for diel and population studies were conducted in this period when intensive sampling was carried out. Conversely, very few surveys were carried out in 2008 as new areas in the far north of

the Maldives were being investigated with little knowledge of where to find mantas. Despite the low number of surveys in 2008, important discoveries such as the re-sighting of a manta 270 km distance from its first sighting were made during this time (Chapter 5). Experiments were planned around what could be achieved within the constraints of running a tourist operation. Some experiments were devised retrospectively and limited by the method by which the data was collected, or the amount of data collected.

The sampling of individuals was biased by the behaviour of the mantas. Mantas which allowed divers to approach more closely tended to be identified first, whereas skittish mantas, which remained around the periphery of the survey site, were unlikely to be identified. Large female mantas were the most tolerant of divers, so there was bias towards these. Longer periods of survey and the presence of more trained observers increased the percentage of mantas identified per survey.

3.6.3 *Volunteer observer training*

All observer divers were trained in the identification technique described in Chapter 4 and reported in Kitchen-Wheeler (2010), and given an introductory briefing on manta behaviour so that observers who were not experienced around mantas would be able to anticipate behaviours and adapt their own so as not to frighten or disturb the mantas. This training was given by two lectures which consisted of the two knowledge development sections of PADI (Professional Association of Diving Instructors) Manta Diver Speciality (Kitchen-Wheeler and Norman 2002). A full survey site briefing was also given including information on expected length of observation period and specific instruction on observation requirements when they varied from simply obtaining details of all mantas observed.

3.6.4 *Data collection media*

The media used to collect data were digital and film stills, digital video and underwater slates. The slates were prepared with manta outlines and prompts for sex, estimated size (disc width, DW) and tail length information which could be added by writing with a pencil whilst underwater (Figure 3.25). Observers were instructed either to obtain photographs or video of the ventral surface of the mantas, or to make notes on a slate of identification criteria, and specific behaviours. Experienced observers were able to use both photographic equipment and underwater slates. After each dive, information was

collated onto record sheets which are of similar layout to the slate. Additional information on date and time of survey, plus any specific comments on behaviour or colouring were also noted. Information was then transferred to a Microsoft Access database. Information on the survey sites including location, date and time of dive, current strength and direction were made in a logbook along with specific notes from observations e.g. animal behaviour and other interesting items. The full method for use of the database for the storage of data is described in Chapter 4. A large photographic and video data set was collated from the hundreds of observation surveys made and these were later reviewed for specific behaviours and activity as investigation required.

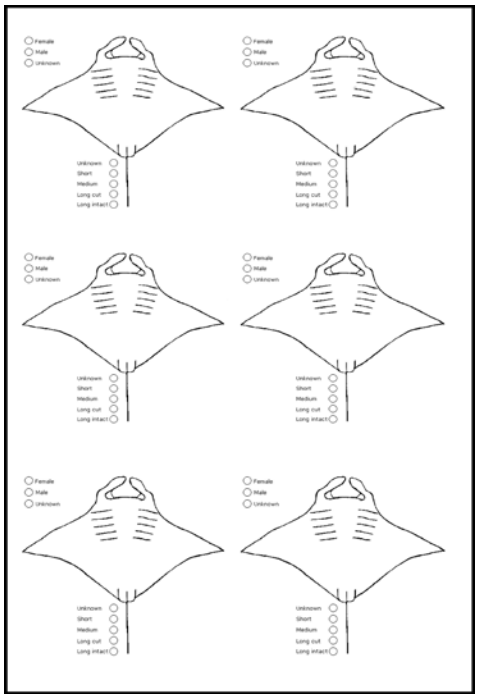


Figure 3.25 The manta identification slate with outlines of mantas as prompts for underwater observation. To the left of each manta outline are prompts for sex (female/male/unknown) and tail length (long intact/long cut/medium/short). Notes are added using pencil. The full methods for determining sex, size and tail status are described in Chapter 4.

Database entry

In 2001-2005 the data were maintained only on paper using both the data sheets described above and a log book. In July 2005 an Access database was designed and all the information transferred to it. Data obtained from 2006 onwards were immediately logged to the Access database. As part of the Access records for each manta, a standardised drawing of each manta's identifying marks was included. This provided a visual record for quick comparison as all used an identical template. Making and use of the standardised drawings is covered in Chapter 4 as part of the manta identification process. To assist in matching new observations to existing identified individuals, a second database of photographs was maintained.

Manta researchers of this species maintain their own databases (G. Stevens, A. Marshall, M. Deakos, F. MacGregor, pers. comms.) and localised populations of *M. alfredi* make the sharing of databases on the species somewhat futile (Clark 2008; Dewar et al. 2008; Deakos et al. 2011). The results of using this database are included throughout this thesis with summary results included in Chapter 4. It would be useful in the future to have a database for the Maldives which could be contributed to, and researched by any interested individual.

Chapter 4. Individual identification of Alfred (*Manta alfredi*) and giant mantas (*M. birostris*) in the Maldives

4.1 Abstract

Knowledge of the abundance of mantas and their migration patterns is essential for conservation, and the first stage in any study is a method to identify individuals. The purpose of this Chapter is to present a method of visual identification of individual manta (of either species) which can be used at any site where mantas are regularly observed. In mantas, each individual has a characteristic pattern of dark markings on the ventral side. The most important individual identifying marks were patterns of ovals in the area between the gill slits, supported by distinctive patterns of mottles and spots in the lower abdominal area (posterior to the gill slits) and areas of dark pigmentation around the head and posterior wing margins. Significant changes in ventral makings were not observed in observation periods exceeding 7 years. Scars and missing tissue may be distinctive for an individual and could be expected to be visible for at least 2 years from first sighting of the injury. The method was designed for identification of chevron colour-type mantas, but is equally useful in identifying black colour-type individuals, and has a wider application for other mantas or marine megafauna taxa including whale sharks and cetaceans. This method was used to identify over 1900 individual *Manta alfredi* and 8 *M. birostris* in the Maldives with the records stored in a Microsoft Access database.

4.2 Introduction

At the commencement of this project, there was very little published information on the distribution or biology of mantas (Yano 1998; Homma et al. 1999). In order to undertake studies on behaviour or migration it was essential to develop a system for identification of individuals. During the period when the investigations were carried out, it was considered that there was only one species of manta but that there were two colour morphs, chevron and black. This method was developed to identify individual mantas observed in the Maldives which are primarily chevron *M. alfredi.* Exceptions observed were initially thought to be an intermediate colour type but comparisons with Kumli and Rubin's (2008) database of *Manta birostris* from the East Pacific suggested that the intermediate colour type were a different species. This species was confirmed to be *M. birostris* (Marshall et al. 2009), indicating sympatry of species in the Maldives.

Methods for identifying individual animals in the field have been developed for other charismatic marine species. There are seven basic techniques for identifying individual fish (Neilson 1992) of which external tags, external marks (cuts or notches applied to the animal by researchers) and natural markings have most relevance in identifying wild animals. Tags are useful with smaller numbers of animals or within a less mobile population when each animal can be individually tagged and then identified from the tag (number or colour). Simple colour-coded streamer (spaghetti) tags were used by Klimley (1983) to undertake population studies of scalloped hammerhead sharks *Sphyrna lewini* (Griffith and Smith, 1834), whilst coded ultrasonic tags were used to monitor the movement of 18 individual sharks (Klimley 1987). A manta tagging experiment was conducted in North-West Ari atoll in the Maldives in early 1996 by dive centre leaders H. Voightmann and N. Schmidt using colour coded "Floy" spaghetti tags (Anderson 1996). Around 50 mantas were tagged, however only one tag was ever re-sighted (R.C. Anderson, pers. comm.) so this method was considered ineffective in identifying individual mantas. An acoustic tagging experiment was conducted on a small number of mantas in 2007 but the tags were lost from the mantas (cause unknown) within a month (G. Stevens, pers. comm.). Based on this latter observation I suggest that the Floy tags in the earlier experiment (Anderson 1996) were also lost.

Identification of individuals from marks (scars, missing tissue and deformities) and natural markings (spots, stripes or other markings patterns borne by all members of the species) depends on specific physical characteristics which show variation within the species. Marks are commonly used to identify individuals within cetacean species. For example, humpback whales *Megaptera novaeangliae* (Borowski, 1781) were identified by the ventral aspect of the tail fluke and left and right aspects of the dorsal fins (Blackmer et al. 2000), and dorsal fin shape (natural variation and damage) was used to identify individuals in bottlenose dolphins *Tursiops truncatus* (Montagu, 1821) (Lodi et al. 2009). In elasmobranchs, marks are used where there is little variation in appearance of individuals. For example, great white sharks *Carcharodon carcharias* (Linnaeus, 1758) were identified from notches and scars on the dorsal fin (Klimley and Ainley 1998), whilst Buray et al. (2009) used a protocol based on natural marks on fins, and coloured spots and scars on the body to differentiate individual sicklefin lemon sharks *Negaprion acutidens* (Rüppell, 1837). Castro and Rosa (2005) investigated marks on nurse sharks *Ginglymostoma cirratum* (Bonaterre, 1788) and found that only 46% of individuals bore distinctive markings. Natural markings (patterns) are more rarely used

to identify elasmobranch individuals and the most commonly reported use is in whale shark *Rhincodon typus* Smith, 1828, which can be individually identified by the spot pattern between the gill-slits and pectoral fins (the most easily photographed area of the animal). An automated spot matching method developed for star pattern recognition has been applied by Arzoumanian et al. (2005) for this species (Figure 4.1).

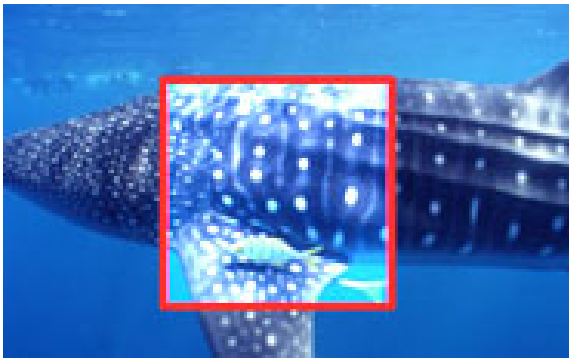


Figure 4.1 One method used to identify individual whale sharks compares the area of spots between the gill-slits and pectoral fins (Arzoumanian et al. 2005). This method standardises the area of spots compared and has been adopted by other whale shark researchers (Riley et al. 2010).

A method was developed to identify sand tiger sharks *Carcharias taurus* (Rafinesque 1810) from their natural markings (Allen and Peddemors 2001) including tears and notches in fins, fin spots and flank spots. Van Tienhoven at al. (2007) specifically used the left flank from the first dorsal fin to the caudal peduncle. A two dimensional affine transformation was used for the mapping onto the common space which considered that each shark was strictly two dimensional. To allow reliable comparison between the shark photographs, reference points of the origins of the two dorsal fins and the origin of the pelvic fins were used and given an (x,y) image co-ordinate. This transformation matrix allowed for automatic comparison of photographs. The problem with using either of these methods for manta individual identification and matching was that mantas are able to distort their surfaces significantly more than a shark might so they are less likely to be considered as two dimensional. At the time of writing a semi-automated identification system using Matlab and the neural network tool box had been developed which has been useful in matching photographs of individual mantas from this database. Essentially it works by clicking the mouse in the centre of all ventral markings (spots, ovals etc.) visible in the photograph, working out the nearest neighbour distances and angles, and processing this information in a neural network. Processing time of each photograph (clicking each spot) is the main constraint, but it is hoped with further development that this will become a useful system (R. Stafford, pers. comm.). There are at least four other projects attempting to develop an automatic identification method for matching the ventral marks of mantas at the time of writing.

The two recognised species of manta are physically quite similar (Marshall et al. 2009), distinguished only by detail which is easily missed if not specifically looked for. This is probably the reason why all previously described species were synonymised under *M. birostris* in the 1950s (Bigelow and Schroeder 1953; Eschmeyer 2008). *Manta birostris* is the larger of the two species, growing to nearly 7 m DW as a large adult, but most individuals seen are smaller than this and *Manta alfredi* grows to almost 5 m DW. Size cannot be used to differentiate species except in individuals larger than 5 m. *M. birostris* has teeth (enlarged dermal denticles) in both jaws, whilst *M. alfredi* has teeth in the lower jaw only but this characteristic is difficult to determine in animals in the wild. *M. birostris* has a postdorsal protuberance behind the dorsal fin, anterior to the tail, *M. alfredi* has no protuberance. Both species have a mainly dark dorsal surface and white ventral surface with characteristic sets of markings on both surfaces. In the majority of mantas of both species, a chevron shaped mark is located on the dorsal surface anterior to the tail and most individuals have distinct 'collars' (*M. alfredi*) or 'shoulder bars' (*M. birostris*) behind the head (Figure 4.2). 'Black' mantas of both species are darkly pigmented over the entire dorsal surface and thus lack distinctive dorsal patterns.

Each individual manta, of either species, has a characteristic pattern of dark markings on the ventral surface. This pattern has been used by Takashi Itoh, a professional diver (Ishihara and Homma 1995; Homma et al. 1999) and Yano et al. (1999a) to identify individual mantas in the Yaeyama and Ogasawara Islands, Japan. In addition, Manta Bay Resort in Yap, Micronesia have kept records of standardised drawings of the ventral surface markings of the mantas which regularly visit cleaning stations nearby (from Manta Ray Resort website, manta ID programme: www.mantaray.com). Acker (2006) has over 100 mantas identified from Yap and stores a photograph of the ventral surface plus a standardised drawing of each manta for reference. Only five of the records are available directly via the website. An example is shown in Figure 4.3. The Manta Pacific Research Foundation (MPRF), based in Kona, Hawaii (MPRF website: www.mantapacific.org) have an online database of around two hundred mantas from Kona and Maui islands. Each record contains data on sex, size and sightings with a ventral photograph (Figure 4.4). Records go back to 1979 however most animals are recorded as being sighted only once. A form to be used by contributors of photographs and sightings data is also available online.

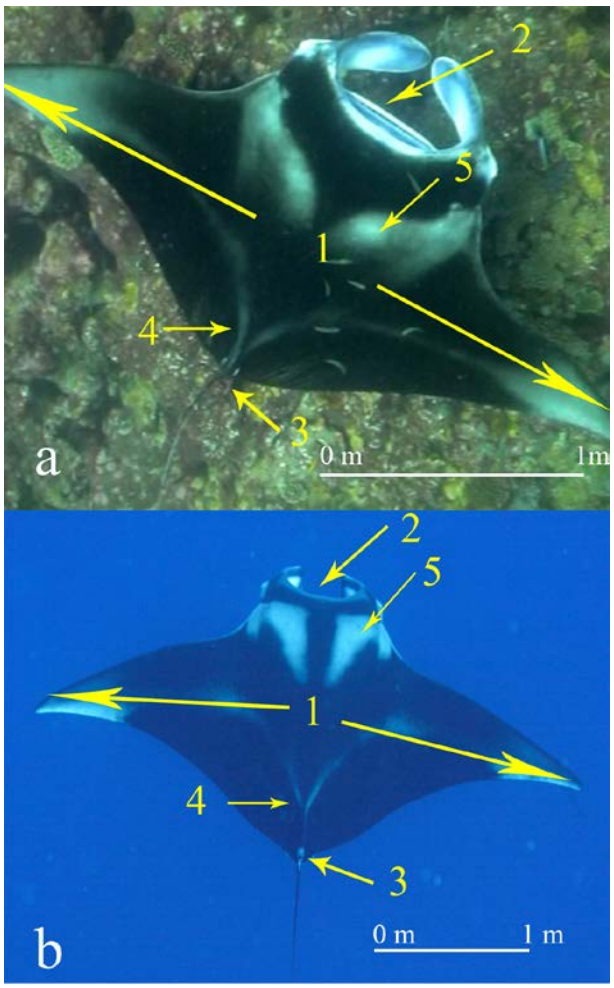


Figure 4.2 Distinctive physical characteristics to distinguish *Manta alfredi* (a) from *Manta birostris* (b). (1) *M. birostris* may grow to maximum disc width of 7 m whilst *M. alfredi* is smaller, maximum DW of 4.9 m. (2) *M. birostris* has teeth in lower jaw and enlarged dermal denticles in upper jaw but *M. alfredi* has teeth in lower jaw only. (3) *M. birostris* has a postdorsal protuberance whilst *M. alfredi* does not. In mantas of both species when a chevron (4) is present a "collar" (5) is seen in *M. alfredi* and "shoulder bars" (5) in *M. birostris.*

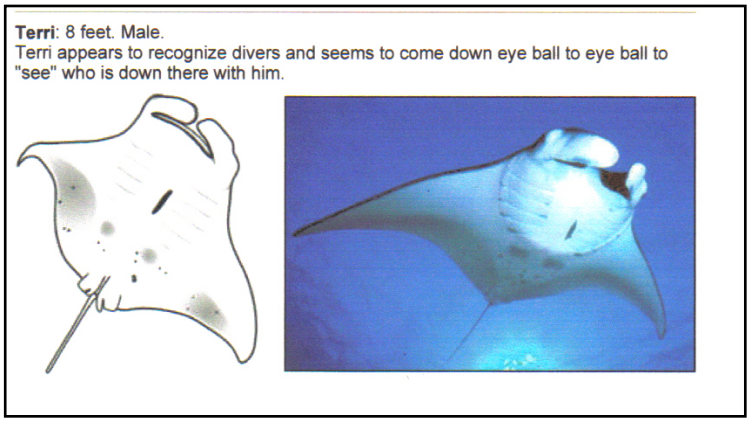


Figure 4.3 Information from www.mantaray.com on manta identification in Yap. The entry (shown here) for manta "Terri" indicates sex, size, and has a photograph and standardised drawing of the ventral side showing unique identifying marks, but no method was given.

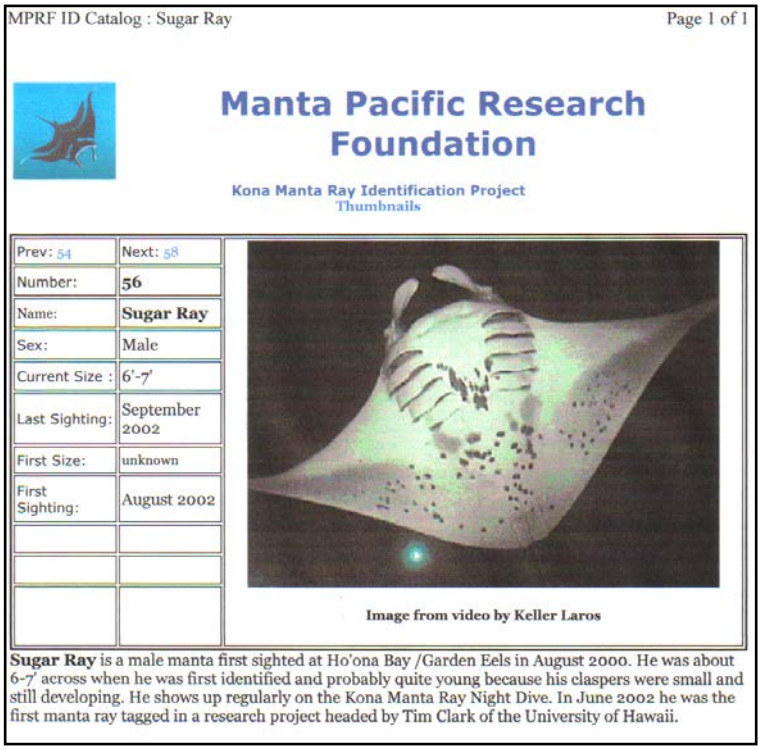


Figure 4.4 Example of Manta Pacific Research Foundation (Hawaii) database entry for an individual manta. The record includes data on sex, size, sighting date plus a ventral photograph.

Both populations are exclusively *M. alfredi*. Although there is a consensus amongst manta researchers that the markings can be used to aid individual identification (Graham et al. 2008; Couturier et al. 2011; Deakos et al. 2011; Marshall et al. 2011), there was no stated systematic method for recording and analysing markings. There was one published international database of mantas available to researchers via the Manta Network (Aston 2008). This was a simple photo record plus description of where the animal was seen with no automated search facility so it was necessary to review each record when searching for an individual. The website included a PDF document called "Field Guide for the Identification of Manta & Mobula Rays" for which the method outlined in this Chapter is used to identify individual mantas and is credited to A-M Kitchen-Wheeler.

The identification method and criteria described below were developed using feedback from identification surveys. Experience (over 200 hours of in-water observations prior to commencement of surveys) had indicated that only the ventral markings were sufficiently distinctive to be used to identify individuals, however general colouration could be used to temporarily identify individuals within a group being observed (e.g. the 'darker' one). Four sets of ventral markings are discussed in this method (Figure 4.5). These are: 1) Marks between the gill-slits (only observed in *Manta alfredi*, in *M. birostris* the marks in the abdominal area posterior to the gill-slits are used to identify

individuals as marks between the gill-slits are always absent); 2) Marks at the distal end of the gill-slits; 3) Marks in the abdominal area posterior to the gill-slits and 4) Areas of extensive dark pigmentation at posterior distal wing margins and around mouth. The methodology described here was reported in Kitchen-Wheeler (2010).

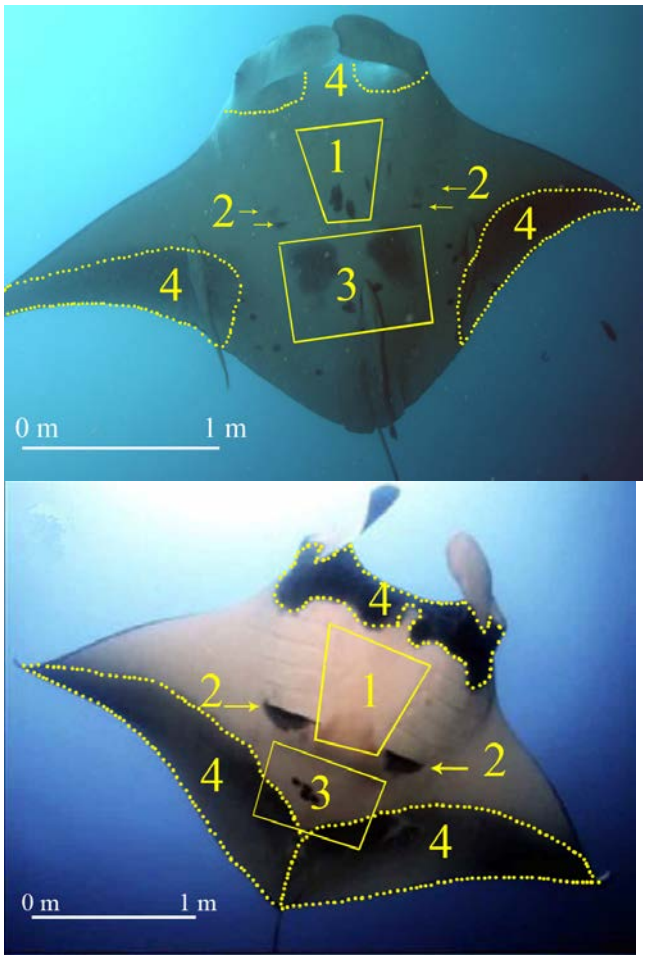


Figure 4.5 Photographs of ventral surface of typical *Manta alfredi* (top) and *M. birostris* (bottom). The four areas where ventral markings can be used for individual identification are indicated. Area 1 (between the gill-slits) **only** contains marks in *M. alfredi*. Area 2 (the distal ends of gill-slits) may be darkly pigmented in both species. Area 3 (the abdominal area immediately posterior to the gill-slits) may contain distinctive marks in both species. Areas 4 may be strongly pigmented and includes the posterior wing margins and around the mouth. Each area is discussed in more depth in section 4.3.

4.3 Method

Between November 2001 and November 2007, 383 surveys were conducted at 8 principal survey sites (which were all cleaning stations, described in Chapter 3) plus 7 less important survey sites where mantas may be observed (14 out of 15 of these sites were located in the central atolls). Each survey consisted of a dive on scuba for a period of approximately one hour to depths ranging between 2 m and 30 m with the specific objective of observing mantas. Data were captured using still cameras (both film and digital), digital video and sketches and notes made on an underwater slate. The latter were necessary due the difficulty in capturing a photographic or video still of the entire ventral side of the manta being surveyed, including the genitalia and tail. All information was collated after the dive and transferred to record sheets and latterly, to

the Access database. This method details the physical characteristics that must be observed and collected to enable positive identification of an individual manta.

4.3.1 *Differentiation of chevron and black morphs*

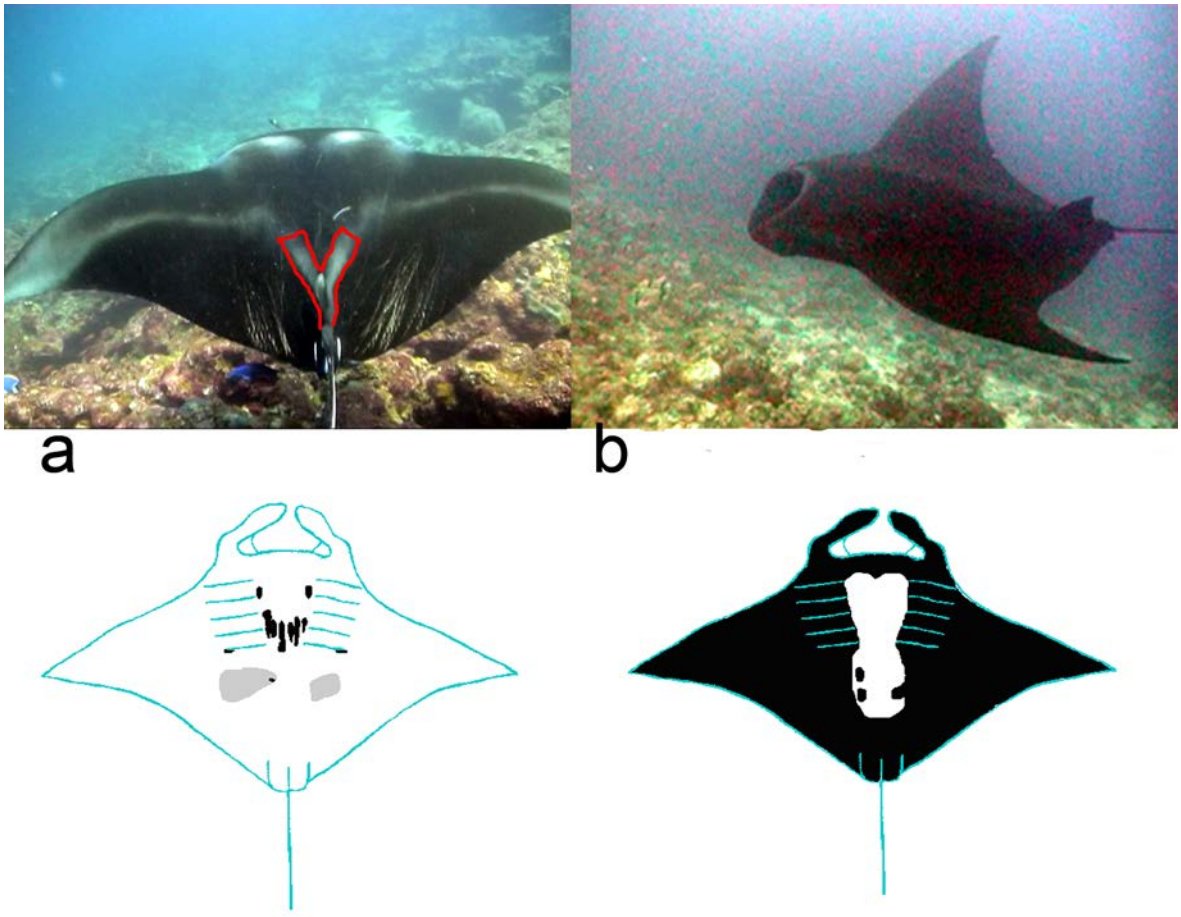


Figure 4.6 Comparison of dorsal (top) and ventral (bottom) colour patterns of typical chevron (left) and black mantas (right). The photograph (top left) shows that the V-shaped chevron (outlined in red) on the dorsal surface extends laterally to the wing tips as a pale stripe. The black manta (right) has an entirely black dorsal surface (photograph top right) and the ventral surface is mainly black pigmented (bottom right). Ventral pigmentation coverage can vary from 50% to 90%. (Left: *M. alfredi*, North Male atoll 15.10.2003; right: *M. birostris*, Ari atoll 4.4.2003).

Two distinct colour variants in both *M. alfredi and M. birostris* have been identified: chevron and black (Barton 1948; Marshall et al. 2009). Chevron mantas of both species have a distinctive V-shaped area of pale pigment on the dorsal surface just anterior to the dorsal fin (Figure 4.6a) and have widely varying dorsal and ventral colour patterns. Black mantas of both species are entirely black on the dorsal side and the majority of the ventral side is darkly pigmented (Figure 4.6b) except for the areas between the gill-

slits and the abdominal area posterior to the gill-slits. These areas are mainly un-pigmented (white) but may contain patches of pigmentation which vary with species as described in section 4.2.2. The vast majority of mantas observed in the Maldives were chevron *M. alfredi* (see results) and the methodology developed here was primarily developed to identify chevron mantas, but can be adapted for black mantas. Distinctive patterns of ovals are clearly visible within the white areas in both species, thus the method described can still be used in black mantas. No black variant of *M. alfredi* has yet been reported from the Maldives but black mantas are commonly reported from Eastern Australia (Frazer McGregor, pers. comm.), Komodo (Rhys Williams, pers. comm.), and Fiji (DIVE magazine February 2011 edition).

4.3.2 *Identification criteria list*

In order to achieve a definite identification of an individual, the following characters should be recorded (see Figures 4.7 and 4.8 for diagram of location of each):

1. Sex.
2. Disc width.
3. State of tail.
4. Pattern of markings between gill-slits* (Area 1).
5. Pattern of distinctive grey mottles and black spots on the ventral surface posterior to the gill-slits*(Area 3).
6. Pattern of distinct dark grey patches on distal ends of gill-slits (Area 2).
7. Degree of pigmentation of ventral surface of wings along distal margins and area posterior to gill-slits (Area 4).
8. Degree of pigmentation around mouth (Area 4).
9. Distinctive dorsal pigmentation.
10. Bites (missing tissue) and prominent scars and scratches.
11. Presence of remoras.

*In *M. birostris* patterns of ovals between the gill-slits are absent: instead of mottles and spots in the posterior abdominal area, patterns of ovals are present. The marks in both areas should be recorded for each animal to ensure identification.

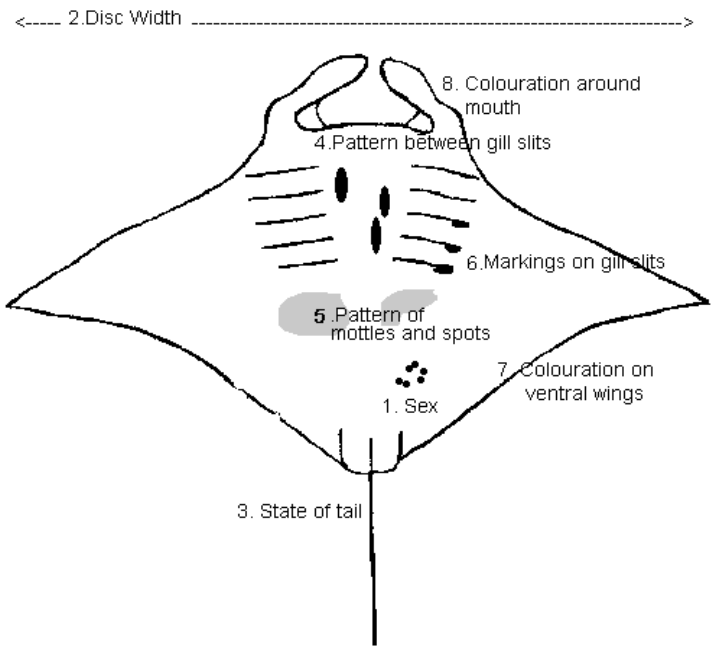


Figure 4.7 Ventral identification criteria.

1. Sex,
2. Size,
3. State of tail
4. Pattern between gill-slits
5. Pattern of mottles and spots posterior to gill-slits or ovals in *M. birostris*
6. Markings on distal ends of gill-slits
7. Pigmentation on posterior wings
8. Colouration around mouth

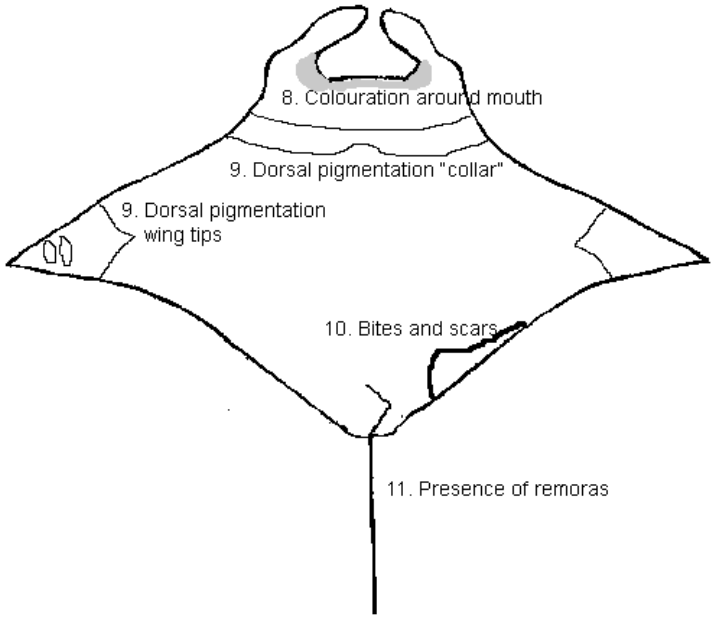


Figure 4.8 Dorsal Identification criteria

9. Pigmentation around head "collar" and wing tips "cuffs" unless *Manta birostris* which has "shoulder bars"
10. Bites and scars
11. Presence of remoras

The first three characteristics can be recorded quickly and should thus be recorded initially. The fourth, fifth and sixth allow progressive narrowing down to > 99% positive identification. The remaining characters provide confirmation. Initially only the first four characters were recorded but these only allowed positive identification of approximately 90% of mantas seen. Adding the fifth and sixth characters enabled all mantas to be identified except those with all-white ventral colouration which have never

yet been recorded. These latter characteristics are also used to identify species. This method can be used for identification of individuals of both species. Each criterion is described in more detail below and variations characteristic for identification of species are identified.

1. Sex

Individuals can be sexed visually. The female (Figure 4.9 left) has a simple cloaca located between the paired pelvic fins: the male (Figure 4.9 right) has two claspers extending from the pelvic fins. In sub-adult males the claspers may be relatively undeveloped and therefore more difficult to detect (Chapter 10 Figure 10.2).

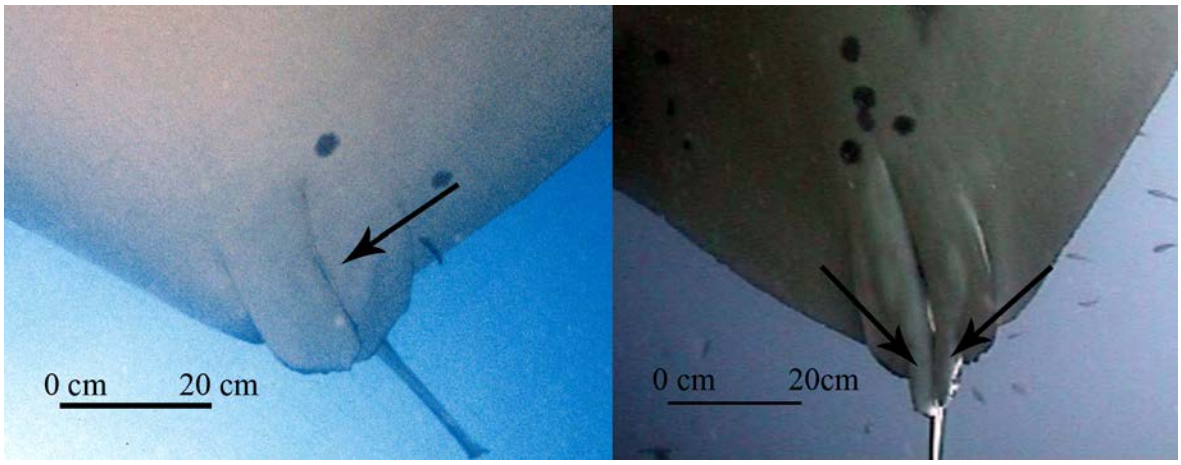


Figure 4.9 Female genitalia (left) show a simple cloaca between pelvic fins (arrowed). Male genitalia (right) shows paired claspers (arrowed) extending from pelvic fins. In juvenile males the claspers are less developed and are more difficult to detect.

2. Disc width

Disc widths (DW) were estimated in proportion to nearby diver size (Figure 4.10). Mantas were very wary of measuring tapes laid on the reef or in the water column so calibrating visual estimates was difficult. Estimation was checked on one survey when a 4 m x 4 m square area was taped off at a cleaning station. Two animals were measured against the taped area on the reef (within ±30 cm) to 4.1 m and 2.5 m wingspan. An additional five mantas were measured using a tape held across the dorsal surface and compared to the estimated size. Measured sizes were 0.1-0.5 m greater than estimates. Attempting to measure mantas caused significant disruption to their cleaning behaviour, so few attempts were made.

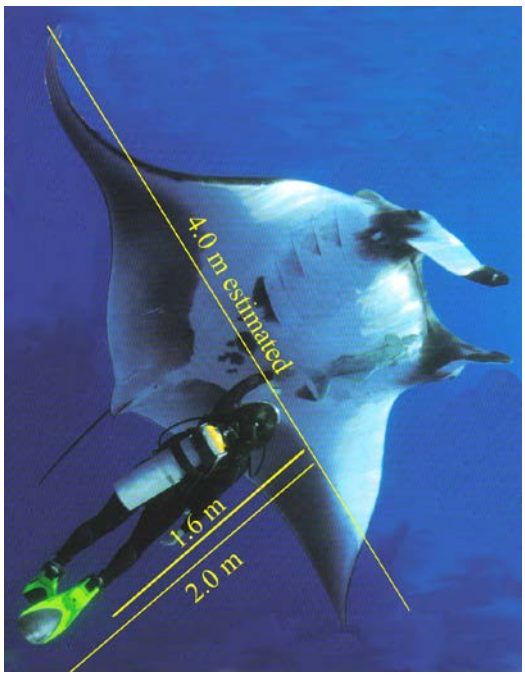


Figure 4.10 Nearby divers can be used to estimate size of mantas. Diver with fins = 2 m, this manta is approx. 4 m DW. (Photograph: Franco Banfi).

Paired laser photogrammetry was used to measure mantas in Hawaii (Deakos 2010). This method required that a photograph was taken of the full length (or width) of the dorsal or ventral side. This method was not used in this study as it would interfere too much with the mantas' natural behaviour and set a bad example to other manta observers as too much pursuing of the mantas, and swimming above them, would be required by the surveyor. For mantas with very few or similar markings, size can be a useful discriminator.

3. State of tail

Many mantas had damaged tails. Four tail states were recorded:

1) Long intact: no evidence of damage, typically ≥1 m length

2) Long cut: the tail end looks damaged – is blunt rather than pointed – but still appears to be at least 75% of the expected intact length (≥0.75 m).

3) Medium: the tail is less than 75% but more than 25% of expected length (approx. ≥0.25 m-<0.75 m).

4) Short: no tail or less than 25% of expected length (<0.25 m).

Manta tails do not re-grow, so a manta first seen with a short tail could not be subsequently be seen with a long tail. A long tail can be bitten off, so re-sightings could be of a shorter tail.

4. Pattern of markings between gill-slits (Area 1)

There is a pattern of dark, **oval or diamond-shaped** marks located in the area between the gill-slits. Mantas are known to be born with a full set of markings (Marshall et al.

2008; Uchida et al. 2008) and individuals' patterns changed negligibly over a period of at least 6 years in this study (see Figure 4.12 for an example). Manta 209 L181 has been recognised from photographs since 1986, so there is evidence that markings do not change over periods of tens of years and can be used for identification during observation periods within this timescale. This is the most important character for individual identification in *M. alfredi*. This pattern solely will distinguish about 75% of mantas. The more markings present, the more likely that the pattern will uniquely identify an individual. Gill-slits were numbered from anterior (1) to posterior (5) and were described with respect to the animal's orientation (i.e. the reverse of how they appear in ventral photographs). The most common marking (seen in 156 out of 1440 individuals) was a single dark oval between the levels of the fourth and fifth gill-slits (Figure 4.13) but there are so many potential positions for even a single mark that this pattern remains the most useful identifier. When reviewing photographs or stills from video, care must be taken to separate oval markings from cleaner fish. A combination of video and sketches on underwater slates is the most useful way of capturing the data. Figure 4.11 a to c shows some examples of progressively more complex patterns between the gill-slits.

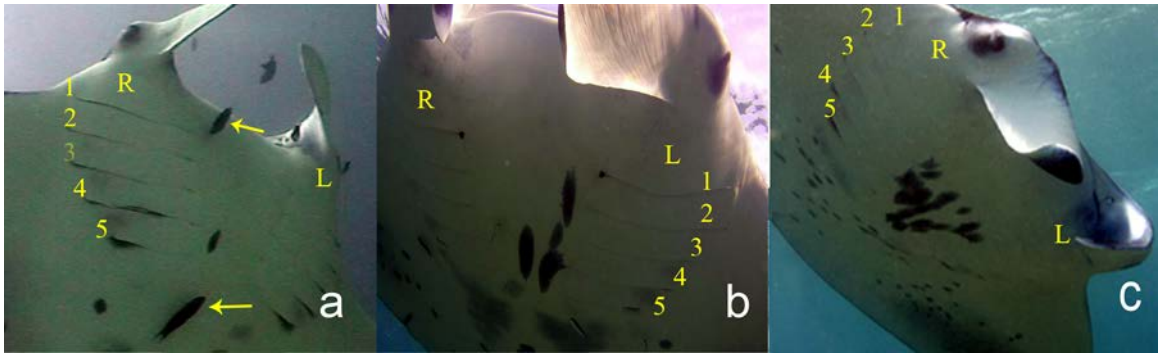


Figure 4.11 (a) has one single oval in Area 1, located between the levels of the fourth and fifth gill-slits. Two oval cleaner fish are visible (arrowed), one near the first gill-slit and one *Thalassoma lunare* posterior to the oval marking. In (b) there is a set of four oval marks of varying size, three are in an approximate line adjacent to the left gill-slits and there is a single oval centrally in line with the fourth gill-slits. In (c) a complex pattern of approximately 19 oval marks create a distinctive 'W', hence this manta is known as Winifred.

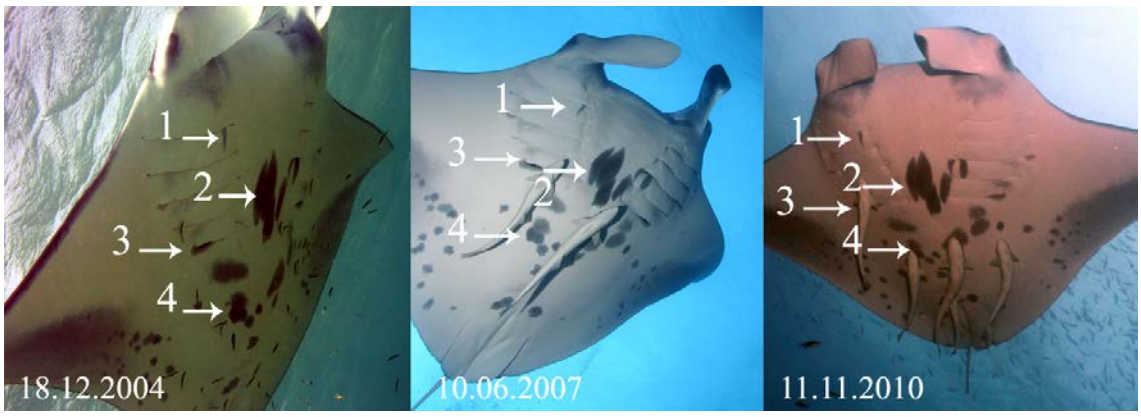


Figure 4.12 Ventral pigmentation and markings vary insignificantly with time. This sequence shows how four markings sets: (1) a small vertical line located under first right gill-slit, (2) pattern of large ovals located between the gill-slits, (3) pigmented end of fifth right gill-slit and (4) pattern of large circular mottles in lower abdominal area; appear the same over a period of six years.

When recording the pattern on a slate, or reviewing photographs or video, it is useful to visualise a notional 20 cell grid using the gill-slits as a frame (Figure 4.13). A very simple pattern may only require an eight cell grid, whereas a more complicated pattern will require more grid cells to record it effectively. An example of how the grid distinguishes a common pattern is shown.

In the first animal (left diagram, Figure 4.14) the mark is in the centre, between gill-slits 4 and 5, but extending slightly upwards from gill-slit 4 level. For the second animal (right diagram, Figure 4.10) the mark is between gill-slits 4 and 5, but is contained within the grid markings, and to the right of the gill-slit area.

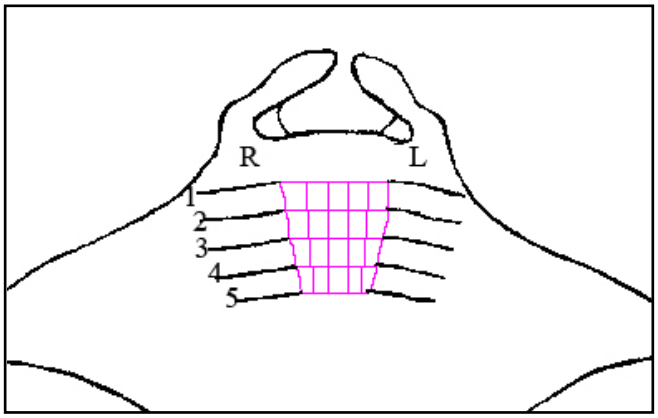


Figure 4.13 Basic 20 cell grid for aiding the placement of marks between gill-slits.

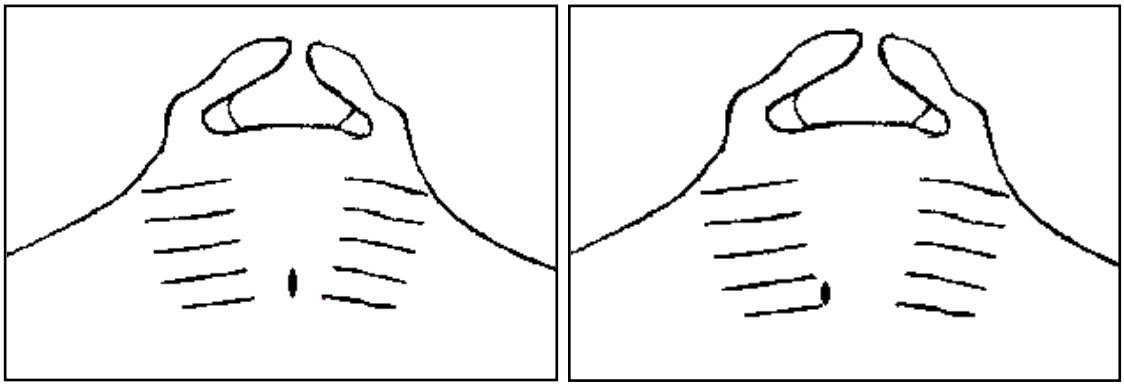


Figure 4.14 Both diagrams of markings have a single oval mark low down in the gill-slit area.

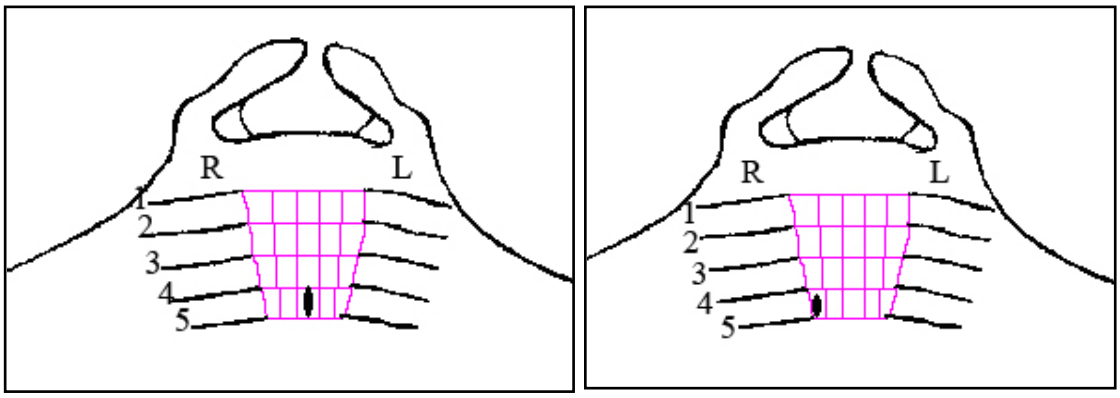


Figure 4.15 If the grid is applied over the diagrams from Figure 4.10 the location of the single mark is seen to be different between the two.

Applying a grid to a more complicated pattern to identify accurately where the marks are will normally result in marks being between sections. For the manta in Figure 4.16 there are five main marks which have been outlined in white to make them easier to identify. The top mark extends outside the grid above slit 1 and the lowest two extend below slit 5. It is common to have marks outside the grid. All five marks cross grid sections. The notional grid helps the observer with accurately positioning the marks.

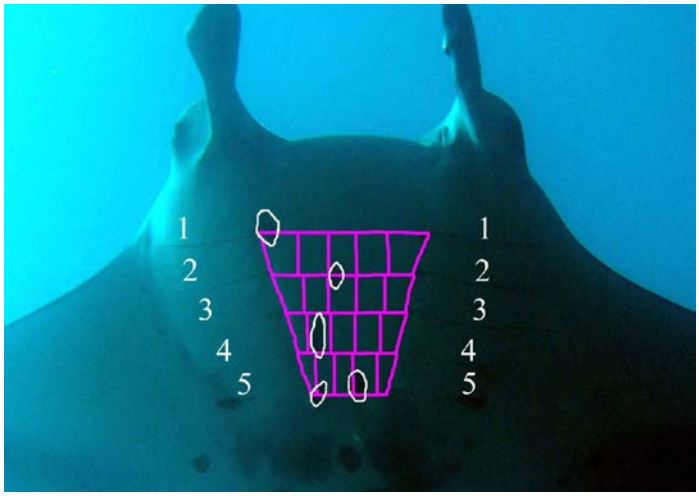


Figure 4.16 Ovals between gill-slits are outlined in white. The notional grid is overlaid to aid description of their positions.

Accurately interpreting the positions of marks between the gill-slits was the main problem with using this method for manta identification because it was difficult to obtain a perfect ventral photograph of a manta. Unlike most animals, mantas are relatively two dimensional, and are thus prone to marked body curvature during their swimming and cleaning routines; this distorts the identification pattern in photographs. In addition, the identification marks are on the ventral area, which is the area least presented to observers. Consequently, the majority of photographs supplied by untrained observers could not be used to identify the mantas due to insufficient capture of the ventral side features. 99 out of 1434 *M. alfredi* observed had no markings between the gill-slits, and all 7 *M. birostris* (a diagnostic characteristic, see Figure 4.47) observed had no markings between the gill so additional markings data need to be collected (criteria 5 to 11).

5. Distinctive grey mottles and black spots on the ventral surface of abdomen posterior to the gill-slits (Area 3)

In individuals of *M. alfredi*, in the area posterior to the gill-slits commencing on the ventral abdomen, there may be pigmentation consisting of grey mottle shading covering between 1% and 80% of the posterior ventral area. The grey mottle may extend towards the posterior wing margins, the wing tips, and anteriorly into the distal spaces between the gill-slits (Figures 4.18, 4.19 and 4.20). Commonly the mottle shading is absent (Figure 4.17). The mottle shading varies in colour from light grey to black. In addition there is often a complicated arrangement of black spots in this area, but in contrast with the marks observed between the gill-slits, the black spots are always circular (diameter 2-8 cm) rather than ovals or diamonds. Exceptions are discussed at the end of this section. This pattern is a very important identifier, especially when there are no or very few marks between the gill-slits. Some examples are shown in Figures 4.17-4.20.

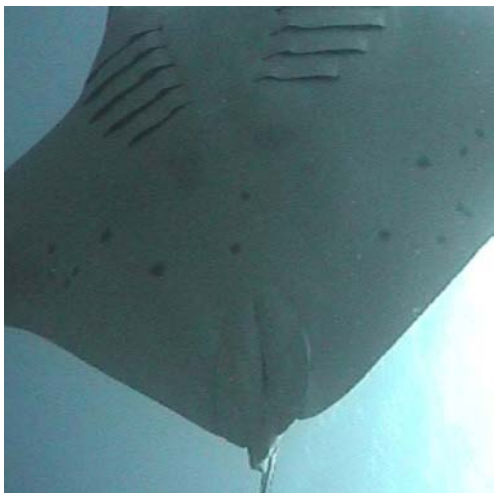


Figure 4.17 Photograph of a simply patterned lower abdomen with no obvious mottles and a few isolated spots (estimated diameter 4 cm). The wide scattering of the spots makes them distinctive.

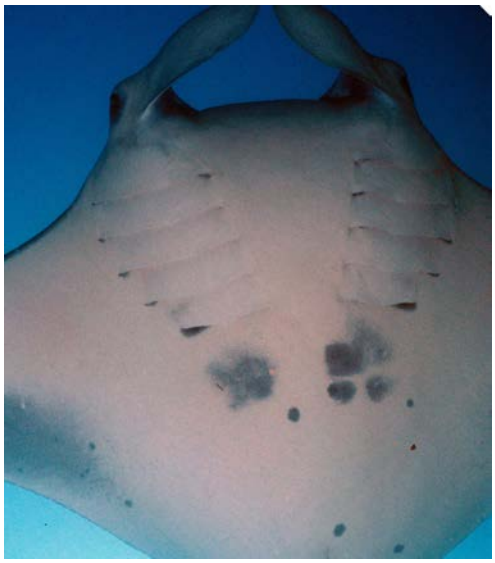
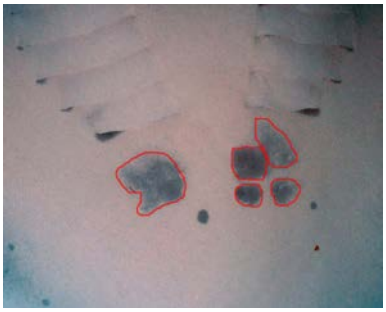


Figure 4.18 There are no markings between the gill-slits of this manta (left), however there are grey mottle areas posterior to the gill-slits (outlined in red below) which are very distinctive.



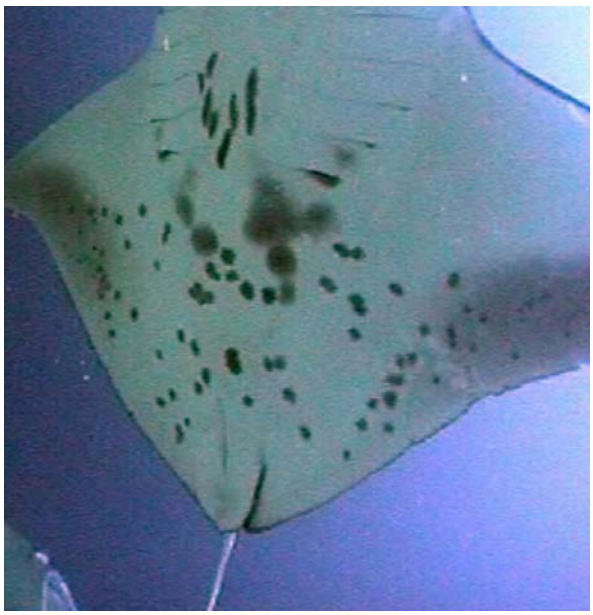


Figure 4.19 This manta (left) has a very complicated set of mottles plus many dark circular spots (estimated diameter 2-7 cm) in the posterior ventral area making it highly distinctive.

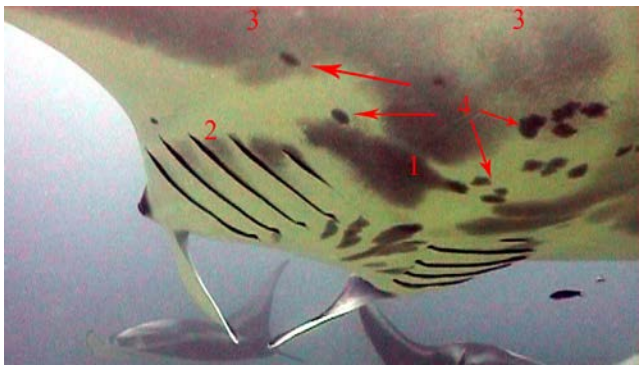


Figure 4.20 This photograph shows extensive dark mottles from the abdomen (1) extending into the distal edges of the gill-slits (2) and across into the posterior wings and lower abdomen (3). Such extensive shading is unusual and thus distinctive. The shading is not continuous and there are black spots dispersed amongst the mottles (4).

The circular spots in the ventral abdominal area can be very distinctive, either in sheer quantity, or in creating specific, eye-catching patterns. Some very distinctive patterns are shown in Figure 4.21.

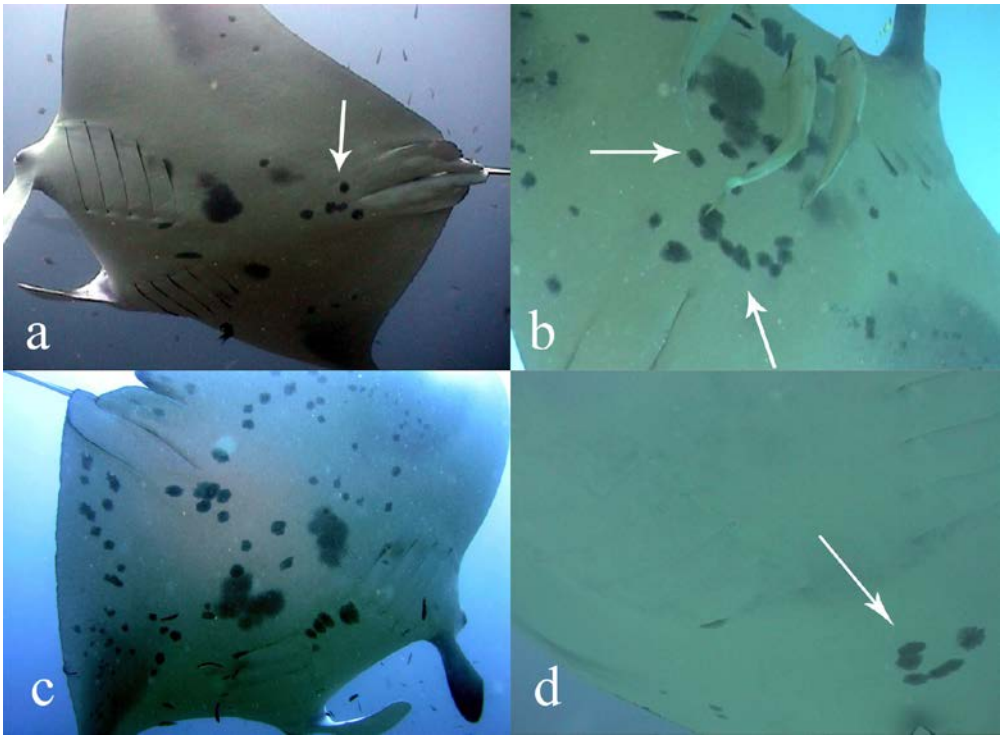


Figure 4.21 Four examples of distinctive spots patterns on ventral surface posterior to gill-slits (Area 3): (a) five spots near genitalia in "Y" shape, (b) complicated pattern of varying sized spots, (c) spots distributed all over posterior ventral area and (d) distinctive six spot set in central posterior abdominal area.

Manta birostris has different ventral markings in the abdominal area posterior to the gill-slits (Area 3: Figure 4.5). Similar to the marks between the gill-slits observed in most individuals of *M. alfredi*, a pattern of ovals, diamonds, and darkly pigmented

mottles (not spots) is present in the **posterior** abdominal area. To identify individual *M. birostris*, use the criteria 1 through 5 and record the number and pattern of marks in the area posterior to the gills slits. Like the marks typically found between the gill-slits in *M. alfredi*, the posterior abdominal ventral markings in *M. birostris* are almost black, and distinctive patterns are formed from combinations of ovals (Figures 4.22 and 4.23).

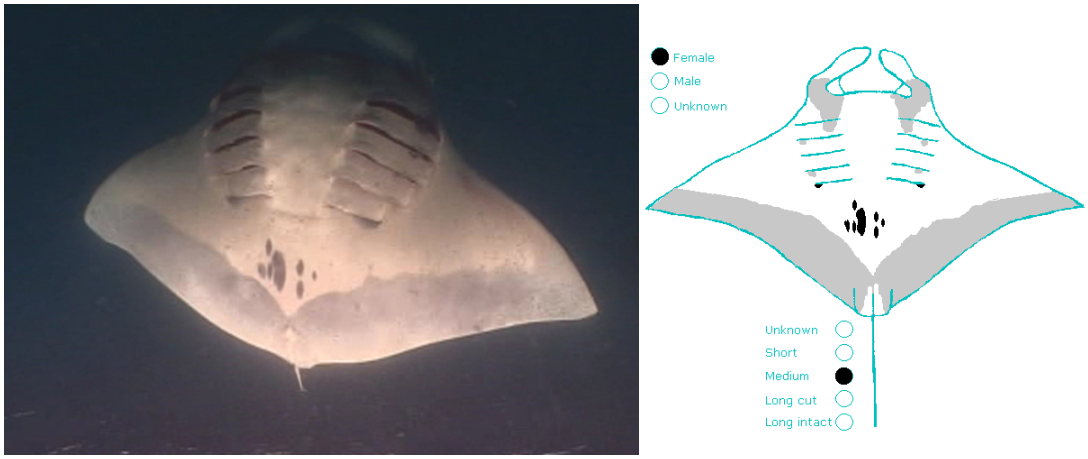


Figure 4.22 Photograph of manta 1452 V11 (left) and its standardised drawing for database entry (right). There are no markings between the gill-slits but there is an area of dark pigmentation posterior to the gill-slits consisting of many oval marks. This manta is identified as *M. birostris.*

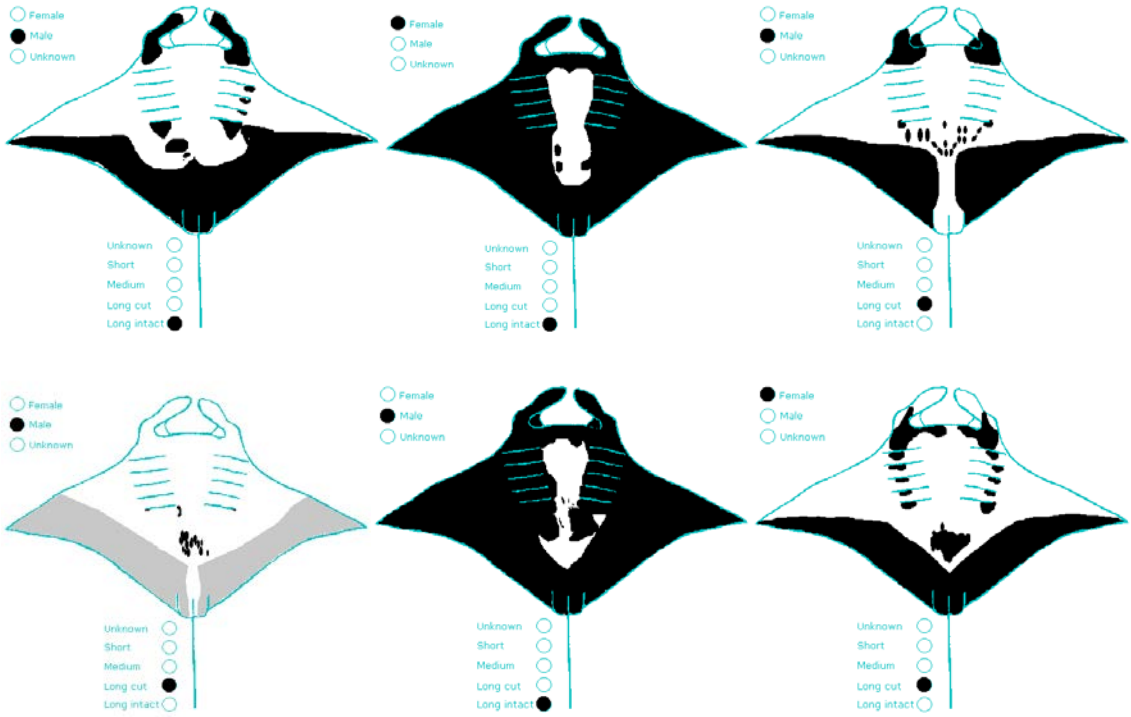


Figure 4.23 Six examples of *M .birostris* from the Maldives database (standardised drawings). All have no markings between the gill-slits and patterns of diamonds, ovals and dark mottles, not circular spots, in the area posterior to the gill-slits.

Photographs of *M. birostris* from locations across the world were reviewed to check conformity. Patterns of ovals or irregular shapes (not spots) were typically found in the area posterior to the gill slits (Area 3) and were always absent from Area 1. In more rare examples the patterns of ovals extended around and into the periphery of the ventral area and towards the anterior and posterior margins. If a manta is observed with no marks between the gill-slits and the pattern in the area posterior to the gill-slits consists of ovals, diamonds and mottles in any combination, with circular spots being absent the manta is likely M. birostris but other physical characteristics should be checked to confirm this.

6. Markings on gill-slits (Area 2)

There may be dark shading on (typically) the distal edges of the gill-slits (Figure 4.24 and 4.25). This shading varies between animals and is present in individuals of both species and is often proportional to the general level of pigmentation of the animal. Thus, a pale animal may have no gill-slit marks whilst a very dark animal may have every gill-slit heavily pigmented. Heavy, bold pigmentation is particularly prevalent in *M. birostris,* see Figure 4.23 for example. The presence of a mark in the gill-slit was recorded as a binary code: 0 = no mark, 1 = mark (Figure 4.26). For each side starting with the gill-slit nearest the mouth the marks were recorded as $1^{st}/2^{nd}/3^{rd}/4^{th}/5^{th}$. If no slits were marked the code would be 00000, if all slits marked11111. The most common combination was for no markings to be recorded (00000, 00000, reported in 48% of *M. alfredi*, 690 out of 1440 records). There were few examples of unique sets, typically non-symmetrical markings e.g. 01011, 00001. These marks were most useful in identifying individual mantas when the marks are visually distinctive due to large areas being pigmented or when non-symmetrical patterns are reported. Routinely, they were used as an objective record of a physical characteristic (see section 4.2.4, Table 4.1). There appears to be wide regional variation of this characteristic based on my own casual review of photographs of both species from other locations around the world. Marshall et al. (2009) described both species as having “semi-circular black spots posterior to the 5^{th} gill-slits” suggesting that dark shading is seen on the distal edges of the 5^{th} gill-slits of <u>all</u> individuals of both species. This study from the Maldives indicates that there is a wide variety in size and position of marks: they may not be present at all (48% of *M. alfredi*) or may be present on a combination (symmetrically or asymmetrically), on any or all gill-slits.

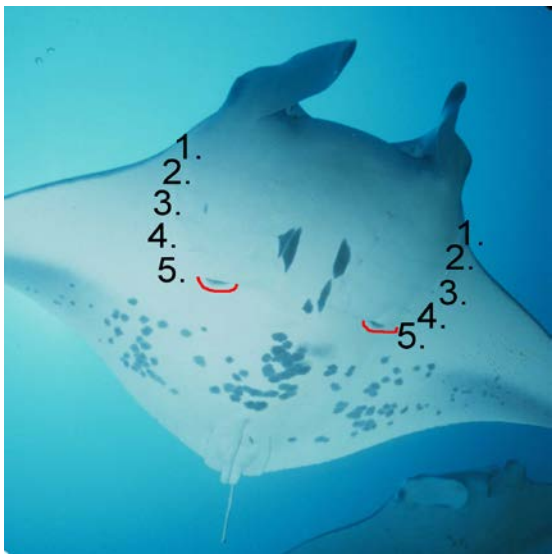


Figure 4.24 Photograph showing black marks on the 5th gill-slit on both sides underlined in red (symmetrical).This is the second most common combination, reported in 31.9% (459 out of 1440) of *M. alfredi*. This would be recorded as 00001, 00001.

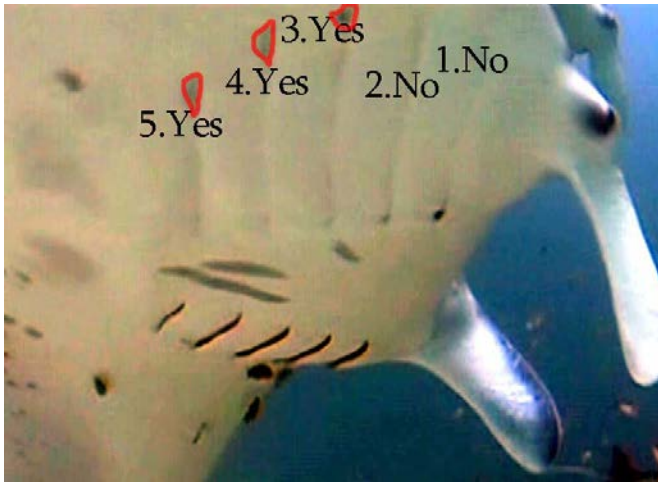


Figure 4.25 Photograph of right-side gill-slits with distal marks outlined. Three gill-slits have pigmentation and thus the right gill-slit markings would be recorded as 00111 for this *M. alfredi.*

7. Degree of pigmentation of ventral surface of wings posterior to gill-slits (Area 4a)

Variations in degree of pigmentation observed on the ventral side of the wings can be characteristic and useful in identifying individuals and species. An area in the posterior half of the wings may be considerably darker than the anterior area. The pattern is an area of increased pigmentation (shading) and is symmetrical from left to right. In *M. alfredi* this is a general indicator of overall pigmentation. Pale mantas will not usually have darkened ventral wings and black mantas will have the ventral wing shading continue anteriorly towards, and including the head, in a single block. Figure 4.26 shows two typical *M. alfredi* examples. The manta on the left has moderate ventral wing shading whilst the example on the right shows no obvious pigmentation.

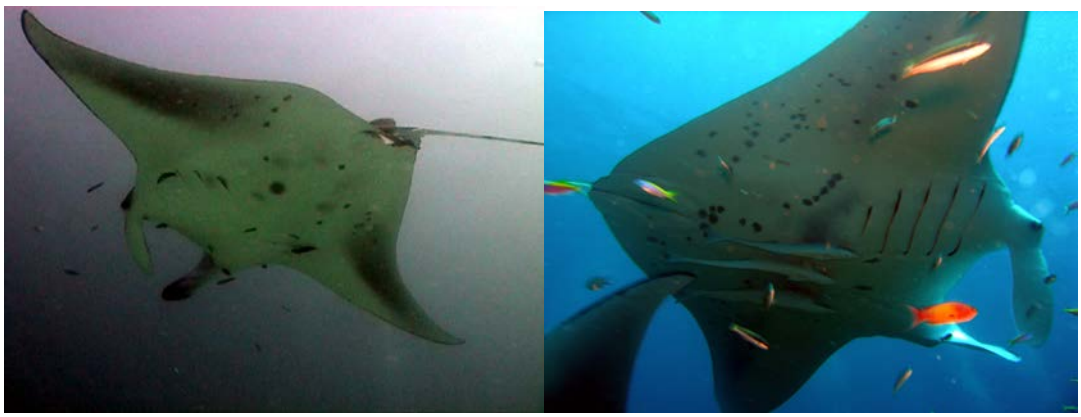


Figure 4.26 Left: *Manta alfredi* with symmetrical posterior wing shading. The shading is not clearly delineated and fades at margins. Right: ventral posterior wing shading is absent.

Individuals of *M. birostris* are very distinctive for this characteristic with the ventral wing area shading being clearly delineated from the non-pigmented area. In *M. birostris*, the pattern and area of pigmentation may vary, but dark pigmentation along the posterior ventral wing margin is nearly always present. At one extreme, the entire posterior ventral area will be heavily pigmented, at the other only the distal ventral wing margin will be (lightly) pigmented, but the pigmentation may also extend to the anterior margin. Figures 4.23 and 4.24 show standardised drawings of the ventral sides of seven *M. birostris* recorded in the Maldives. The ventral wing pigmentation is highly delineated in each example, but colour and area of pigmentation vary considerably between individuals.

8. Degree of pigmentation around mouth (Area 4b)

Figure 4.27 The manta on the left is completely white around the mouth. This is the most common colouration for *M. alfredi* in the Maldives. In contrast, the *M. birostris* on the right has pigmentation extending from the mouth to the eyes creating a 'hood' effect, but the cephalic fin tips remain white.

In both *Manta alfredi* and *M. birostris* the degree and shape of the area of pigmentation around the mouth can be quite distinctive and indicative of the overall level of pigmentation in the animal. In some animals the cephalic fins are also darkly pigmented. Examples of a manta with no mouth pigmentation and one with distinctive 'hood' shaped pigmentation are shown in Figure 4.27.

9. Distinctive dorsal pigmentation

Dorsal pigmentation varies significantly in chevron mantas with variations creating contrast between dark base colour and pale patterns. The area most likely to be pale is the area behind the head and extending to the eyes, which is referred to herein as a 'collar', and the shape appears to be distinctive for *Manta alfredi*. Dorsal pigmentation can vary from black, with shades of purple and brown, through grey and fawn, to completely white. Distinctive dorsal patterns in *M. alfredi* may also include white wing tips; and white shading extending forward from the chevron to the central dorsal surface, and across to the wingtips and the 'collar'. Figure 4.28 shows the chevron, collar and pale wingtips of a chevron *M. alfredi*. There is a faint pale line connecting the chevron to the wingtips. The dorsal pattern varies considerably between individuals and the 'base' or darker dorsal pigmentation varies from black through to pale grey or fawn. Some *M. alfredi* are very dark with only the chevron being pale, whilst others are much paler than the examples in Figure 4.29. The very pale form was referred to as "leucistic" by Marshall et al (2009) and considered rare, but this form is quite common in the Maldives. Chevron *M. birostris* have distinctive pale 'shoulder bars'. These are more angular than the 'collar' observed in *M. alfredi*, and have a wide gap between them (Figure 4.30). Black (melanistic) mantas are entirely black on the dorsal surface in either species. In chevron mantas, the dorsal surface pattern varies considerably between individuals, but is much less distinctive than the ventral pattern. Therefore, records of dorsal pigmentation tend only to be made when it is particularly distinctive, for example unusually pale (Figure 4.26), or entirely black.

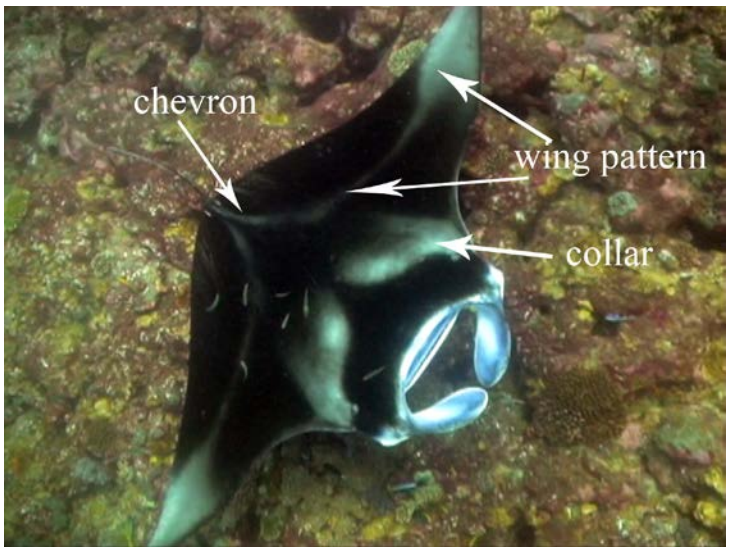


Figure 4.28 *M. alfredi* with the "V" shaped chevron indicated. The collar and wing tips on this manta are pale grey to white. A grey line extends from the chevron to the wing tips. The base colour is very dark grey, almost black.

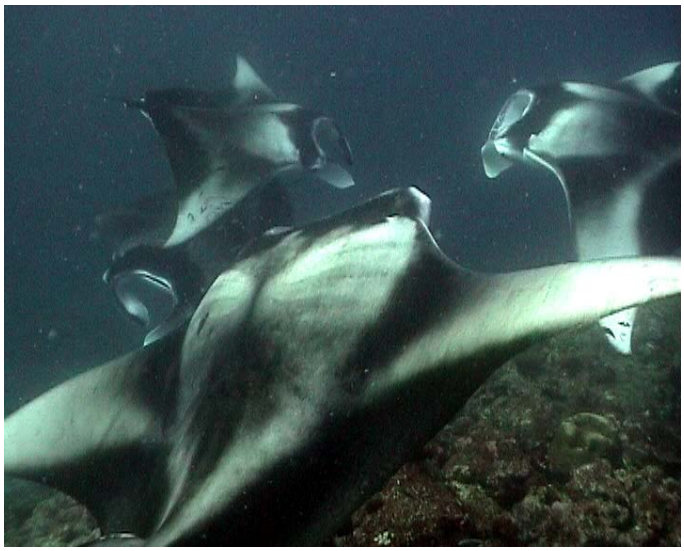


Figure 4.29 A group of *M. alfredi* with variation in dorsal colouration between individuals. The animal in the foreground has a relatively high amount of white compared to the animal in Figure 4.28. All the mantas in this photograph have an almost black base colour.

Figure 4.30 *Manta birostris* dorsal surface. The arrow indicates a gap between the pale shoulder bars. The angular shape of the bars and the gap are distinctive for this species. Photograph courtesy of John Rochester, taken at "Tower", Red Sea.

10. Bites (missing tissue) and prominent scars and scratches

Typically, parts of the posterior wings or cephalic fins were missing or showed damage caused by shark bites (deduced from shape of bite or scar). These marks can be very distinctive. When the damaged tissue is still present, it is often thin in appearance and includes notches and tears. Some examples are shown in Figures 4.31 to 4.33. Superficial scars and scratches were observed healing over 6-12 month periods so were

less useful for identification. Other common scars include mating marks (observed on the left wing tips of mature females and discussed further in Chapter 10) and abrasion scars (the origin of which is unknown, but may be caused by trauma). Whilst superficial scars normally heal in less than 12 months and are not useful for long term identification, larger scars and areas of missing tissue are visible for many years. See results section for analysis.

Figure 4.31 Large section of posterior left wing missing (from possible shark attack) on manta 540 M41. Sightings from 13.2.2003 to 18.3.2009 report same tissue damage.

Figure 4.32 The yellow arrows indicate scarring on left wing tip of female. Most adult females have some form of scarring on the left wing tip (only). The likely cause is from mating where the male clasps the female's left wing tip in order to hold her in position to insert his clasper (Yano et al. 1999b). Mating scars usually heal in around 12 months.

Figure 4.33 This manta has a large, white, permanent abrasion scar on the dorsal side, just above its (missing) tail and blending into the chevron. The posterior left wing, approximately 30 cm left from the missing tail, bears significant scarring from a presumed shark attack. This manta is easily recognisable from its scars.

11. Presence of remoras

Based on review of photographs of *M. alfredi* from eastern Australia and Mozambique (Marshall et al. 2009) and as observed in the Maldives, it appears that *Echeneis naucrates* (Linnaeus 1758) is the remora commonly seen in association with *M. alfredi*. *Remora remora* (Temmick and Schlegel, 1850) was seen in association with *M. birostris* based on assessment of photographs from Revillagigedos islands, Ecuador, Madeira, Hawaii and St. Paul's Rocks (Luiz et al. 2009). In areas of sympatry of manta species, the same pairing of manta species and remoras was observed (A. Marshall pers. comm., pers. obs.).

Although individual remora (*E. naucrates*) which attach to mantas can come and go, once a manta has remoras in association they seem to remain with that individual. This is suggested by the observation that the same numbers of remoras tended to be recorded on subsequent sightings of a particular manta. This may be due to the feeding behaviour of the manta (R. Rubin, pers. comm.) but is not investigated further in this study as the numbers of remoras were not routinely recorded until after 2008 and the dataset was very small. The majority of mantas do not have remoras with them, thus the presence of a large number of remoras is distinctive.

4.3.3 *Field notes*

Identification criteria 1 to 4 were found to be essential for any positive identification and any record with less than 3 of the essential criteria was not added to the database. The most important data required was an accurate record of the markings between the gill-slits (criteria 4). Animals often had to be omitted if a full identification set could not be recorded through photos and notes. The criteria list was added to and refined as experience in matching increased but finalised within 3 months from the commencement of data collection.

4.3.4 *Database entry*

Entries were made following each survey. The database has three forms for data entry 1) Site information, 2) Manta details and 3) Surveys. The Site information form was completed first (Figure 4.34) as information contained in this form was required for other forms. Each site was allocated a site letter. In the example in Figure 4.34 is Lankan site which was allocated the letter L. Once a site was added it became available on drop down lists on the Manta details and Surveys forms.

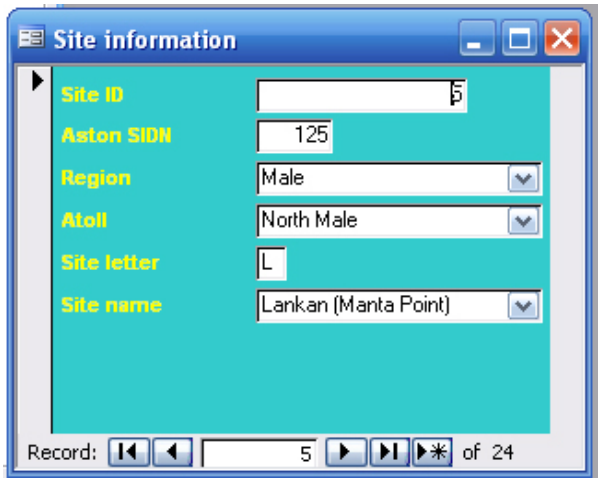


Figure 4.34 A site information form was created for every site. In this example it is for Lankan which was allocated the letter (L) which was used to name all mantas first seen at this site. It also includes additional information on region, atoll (Table 3.2) and information for cross referencing with other databases (SIDN).

The second form to be completed was the Manta details form which contains the following information on each manta: sex, size, tail length, a standardised drawing with identifying marks, quantitative summaries of selected markings, and records of each sighting. Any other relevant information e.g. whether sighted pregnant, relationship with other mantas, courtship etc. were recorded in the comments box. Each individual manta was allocated an alphanumeric code when it was first sighted (Figure 4.35) which derived from its order of sighting, plus a reference to the site where first seen (from the Site information form, Figure 4.34). The manta details form contains general information about every manta in the database. Once a manta was added its code became available in a drop down list on the Surveys form.

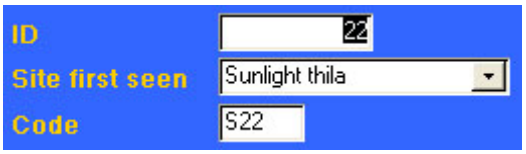


Figure 4.35 Detail from Manta details form. A code of S22 would be allocated to the 22nd manta identified at Sunlight Thila (site S) by the operator. The database automatically allocates a unique "ID" number for the order of entry into the database, this was the 22nd manta in the database and its full ID record number is 22 S22.

The next section on this entry form (Figure 4.36) is the summary of its physical characteristics which could be used in queries to find specific animals with these characteristics. Note the areas listed here do not match the areas listed in the criteria. Area 1 and Area 2 on the database refer to the number of marks in the upper and lower parts, respectively, of what has been referred to as Area 1 in this Chapter and are simply a count of the number of marks present. Area 3 and 4 in the database refer to the

numbering on the right and left gill-slit marks respectively. The database was created before the definitions of the ID criteria were made.

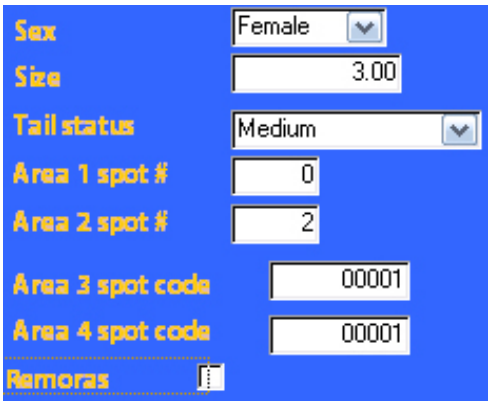


Figure 4.36 Part of Manta details form summarising physical characteristics and markings. Data on sex, size, tail status and number of markings within 4 designated areas were used to search for a specific individual or individuals with the same characteristics.

A comments box allows information on dates sighted, colour, scars, behaviour and changes in physical characteristics to be recorded, e.g. shortening of tail (Figure 4.37).

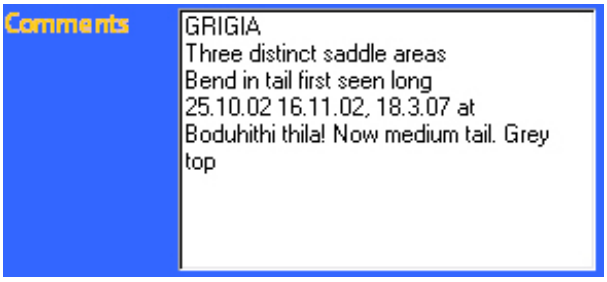


Figure 4.37 Comments box in Manta details form. This includes basic sightings details (stored in more detail in Survey form) and comments from observations.

Once each manta from a survey had been identified, a Survey form to summarise all the information was completed. The survey form includes information on the site (from the Site information drop down list), time of day and summary of how many mantas were seen (Figure 4.40). Within this form every manta identified during the survey was also recorded: mantas identified could be selected from the Manta code drop down list which connects to Manta details. In the example in Figure 4.40, this is the 39th survey recorded (bottom left) and it was conducted at Lankan on 08/11/2003. During this survey it was estimated that 30 mantas were present (top right) but only 26 were identified (lower-centre), and the form is showing the 2nd identified manta entry which is for L20 (upper centre-left).

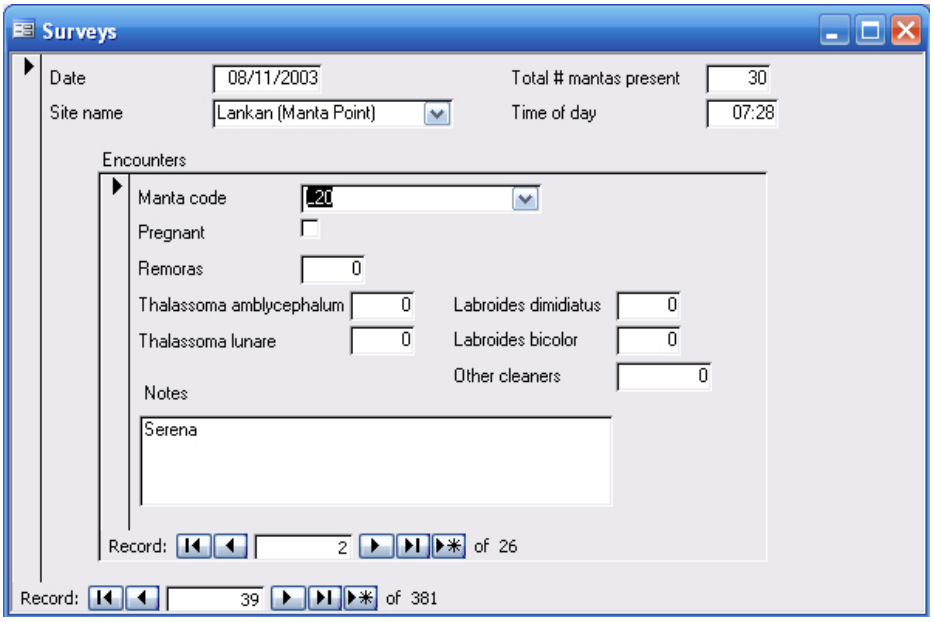


Figure 4.38 The Surveys form was completed after all mantas from a survey had been identified and recorded. Each manta encountered per survey was listed within the Encounters sub-form. Additional data within the Encounters sub-form on cleaners, remoras, etc. were not routinely recorded, but only sought on a few surveys made specifically to record these. In the example above there were 26 encounters recorded (i.e. 26 mantas identified) out of 30 mantas present.

4.3.5 *Matching*

In practice, when the data from a survey was processed, notes and photographs for each manta were collated. To search for a match to a previously identified manta involved the use of queries. The search was refined by entering all known details on sex, tail length and number of marks in the specific areas 1, 2, 3 and 4 into a query to refine the search. Typically 10-30 records were matched automatically and the standardised drawings on the Manta details form were checked for the best visual match. A final check was made using photographs. The drawings on a 500 x 500 pixel GIF format standardised base outline (Figure 4.39) were the main visual record. Photographs and video records were used in support. This method worked very effectively as comparing standardised drawings was easier than comparing photographs. The standardised drawing included a record of sex and tail length next to a sketch of the markings between the gill-slits, distal gill-slit marks and identifying marks in the posterior abdominal area, plus distinctive scars or missing tissue. The drawings were intended to help in matching records, not provide a perfect drawing of the ventral markings. The standardised drawings were amended when new information is obtained; for example, a new photograph or new video footage was obtained and a minor detail required change. In general the standardised drawings were easy to recognise and other observers in

Maldives were easily been able to match the drawings to their own sightings and photographic records.

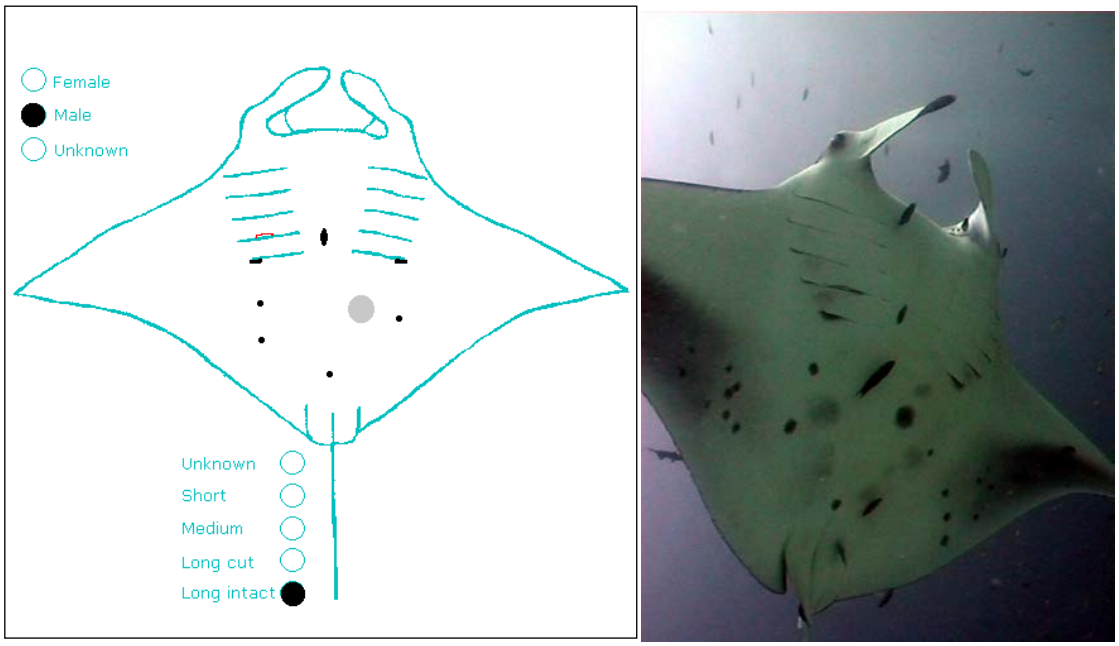


Figure 4.39 Standardised drawing of manta 35 S35 from Access database (left) and file copy of good ventral photograph (right). Only characteristic and obvious marks were copied from photo to standardised drawing. The standardised drawing includes the single oval, slightly right of centre, between gill-slits 4 and 5; four black spots located in the central lower abdominal area; plus a grey mottle left of centre on the abdomen. The red line indicates a scar on the right fourth gill-slit.

When an animal was matched a note of the date of the re-sighting was made along with any additional relevant information including behaviours or pregnancy. An example of the matching process is given in appendix B. If no match was found a new entry was created (Figure 4.40). Certain allowances were made for criteria which might change (i.e. size and tail length) depending on the time between observations. A match of markings between the gill-slits and sex was essential and considered the most useful identifier. It was noted that some spots darkened over time, especially when the mark had originally been recorded as being grey or pale and a period in excess of 12 months had occurred between observations. There was no record of marks disappearing. Size (DW) was reported to increase in some mantas (see results). The matching process was usually performed after each survey, but was sometimes performed several weeks after the survey, especially for sets of data provided by other researchers. During the project, with about 30 minutes of instruction, some 60 assistants had been trained to use the database and search for matches.

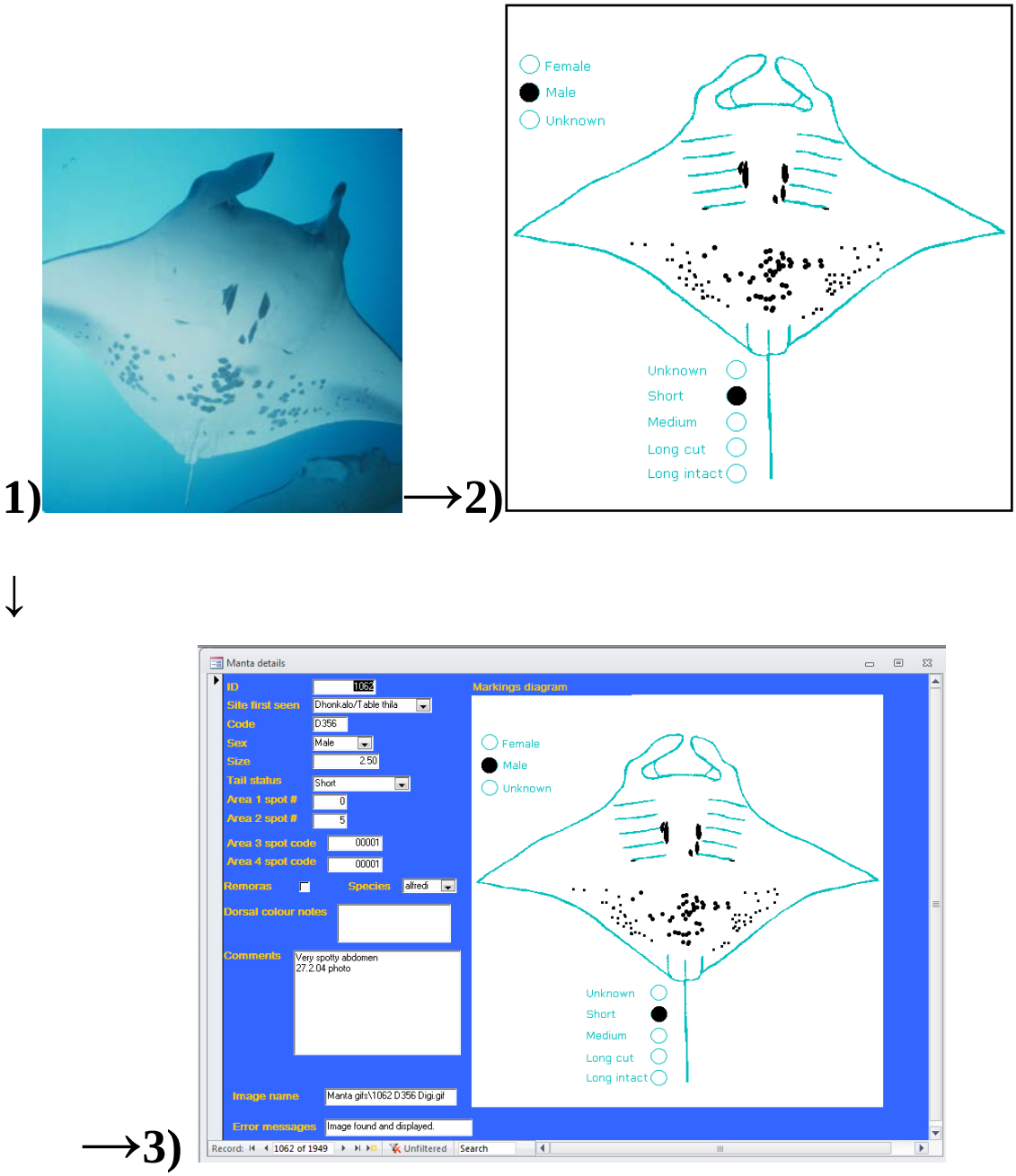


Figure 4.40 Process by which information from a photograph or sketch 1) is transferred to a standardised drawing 2) and then linked to the Access database via the Image name field 3) with other information about date of sighting, survey site, etc. The two unique references for this manta are 1062 (ID) and D356 (Code).

Over the years some duplicated Manta details records were created in error (estimate around 5%). Second (and even third) records were amalgamated with the first record when the duplication was noticed and the database number of the duplicate re-allocated to a new manta. It was important to update Survey forms with updated manta details. Creating a duplicate record presented no problem as the duplication rarely lasted longer than three months as it would be picked up on database reviews and when searching for

matches. Table 4.1 (results) lists the largest and smallest results set for any criteria which could be searched automatically using the Query function. Although marks between the gill-slits will identify a large number of unique markings sets (immediately identifying the manta), a typical search would identify around 20 mantas which required visual assessment of the standardised drawing and file photographs before a match could be made, or a decision taken that a new manta had been observed. Size and tail lengths were not used in initial searches as measures were not sufficiently objective. These criteria would be used for final confirmation as size (DW) could only remain the same or increase with time and tail length remain the same or decrease.

4.4 Results

4.4.1 *Summary database results*

The database was analysed using queries on Manta details to examine different criteria. Only the top-line results which represent the population sampled are shown in this section as the database was also analysed in more detail (e.g. site and individual manta level) in all the following Chapters. It continues to grow at a rate of around 200 new manta additions each year, so for the purposes of this Chapter, the database was investigated as of 7.11.2007 (many additions between 2007 and 2010 were from new sites with low numbers of mantas recorded per site). Table 4.1 lists the largest and smallest samples that were obtained from each of the criteria. The Table shows that sex, size and tail-length have low uniqueness whilst marks between the gill slits and marks at the distal end of gill-slits have high uniqueness and were useful for searching for a specific manta.

The number of individual mantas positively identified during any survey ranged from 1 to 40 animals from a sample of 1 to over 100 animals observed. Table 4.2 lists survey results for each site. The total number of positive identifications was 2449 from 3257 animals sighted identifying a total of 1440 individual mantas. North Male atoll mantas (those first reported at: Lankan, Sunlight, Boduhithi, Fairytale, Aquarium) were encountered a mean of 2.16 (SD ± 2.56) times (698 individuals from 1516 positive identifications, range 1-27 encounters). Ari atoll mantas (those first reported at Table thila, Kalhahandi, Madivaru, Rasdhoo, Ukulhas) were encountered a mean of 1.27 (SD ± 0.82) times (730 individuals from 921 positive identifications, range 1-8 encounters). Subsequent encounters could be at any site. Less than 1% of re-sighted mantas were

reported in a different atoll from first sighting. A full analysis of re-sightings is made in Chapter 5.

Criteria	Largest sample	Smallest sample	Evaluation of criteria
Sex	908 (female)	514 (male)	Objective Low uniqueness
Size	487 (3.0 m)	1 (five unique accurate measurements e.g. 4.1 m)	Subjective as most measures were estimates. Low uniqueness
Marks between gill-slits	156 (0/1)	1 (29 unique marking sets e.g. 0/11, 8/4, 3/11)	Objective High uniqueness
Marks on distal ends gill-slits	691 (00000,00000)	1 (15 unique non symmetrical sets e.g. 01011, 00001)	Objective High uniqueness
Tail length	419 (Long cut)	298 (Long intact)	Moderately objective. Low uniqueness

Table 4.1 List of criteria used in matching with largest and smallest sample size obtained from query of each in database. The final column evaluates using each criterion in a search for a match.

Survey site	Locality (atoll)	Depth range where mantas seen	Number of surveys performed at site	Number of mantas first reported at this site	Number of re-sightings of mantas first seen at site	Total number of observations at site
Lankan	N. Male	15-20 m	174	408	617	1028
Sunlight	N. Male	14-18 m	12	42	107	149
Boduhithi	N. Male	2.5-5 m	47	235	94	329
Fairytale reef	N. Male	18 m	4	8	0	8
Aquarium & Kani	N. Male	15 m	1	2	0	2
Nelivaru	Baa	12-22 m	3	1	0	1
Table thila	Ari	16-22 m	52	510	95	605
Kalhahandi huraa	Ari	15-20 m	15	30	1	31
Madivaru	Ari	12-15 m	71	187	95	282
Ukulhas	Ari	20 m	2	1	0	1
Rasdhoo	Ari	15 m	2	2	0	2
Guraidhoo	S. Male	3-15 m	1	1	0	1
Maavaru	N. Nilandhe	15 m	2	1	0	1
Fushifaru	Lhaviyani	12 m	2	4	0	4
Mudakan	Addu	22 m	2	5	0	5
			390	1440	1009	2449

Table 4.2 Table summarising site, survey and manta sightings data in Access database on 7.11.2007

The most mantas were first reported from Table Thila site (510), but individual mantas first reported at this site were only seen an average of 1.19 (SD ± 0.64) times. There were 411 mantas first reported at Lankan and sighted an average of 2.52 (SD ± 2.99) times. These data sets are non-normally distributed so cannot be compared directly. The apparently higher number of re-sightings from Lankan may simply be due to the higher number of surveys carried out there (174 vs. 52). This is investigated further in Chapters 5 and 7. The number of sightings of individual mantas varied from being seen only once (1088 animals, 75.5%) to 27 sightings of manta 253 L218 known as “Butterfly”.

During the five year observation period the majority of animals were seen only one or two times and only 75 of 695 (10.79%) of North Male atoll based mantas were seen on five or more occasions (range 5 to 27). Of Ari atoll based mantas only 15 of 730 (2.05%) were seen on five or more occasions (range 5 to 8). Five or more sightings would suggest that the manta was seen at least once a year during this period. These overall results are shown in Figure 4.41.The relatively low rate of re-sightings is investigated in Chapter 7.

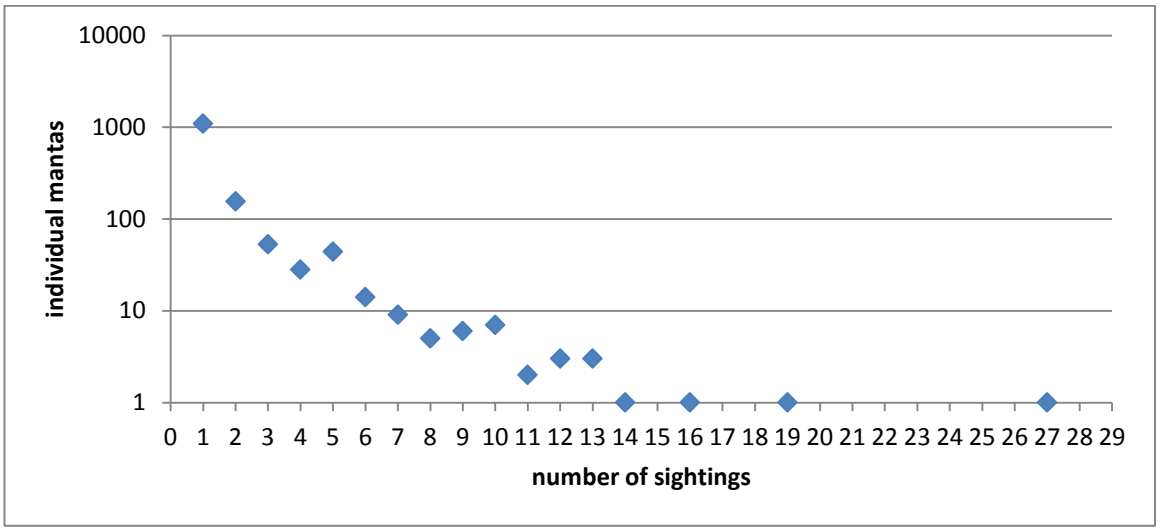


Figure 4.41 Frequency distribution of number of sightings. Individual mantas were sighted between 1 and 27 times. 1088 mantas were seen only once and relatively few were seen five or more times.

4.4.2 *Review of gender mix of mantas observed*

Sex of manta was recorded as Male, Female or Unknown. Only the data of 1422 mantas of known sex were analysed. 514 (36.1%) were recorded as male, 908 (63.9%) were recorded as female. This indicates a strong sex bias in the observed population (χ^2= 109.2, DF = 1, $P < 0.001$) with a ratio of approximately 1:1.8 males to females. The sex

ratio per site is summarised in Table 4.3 (excluding sites where < 10 animals encountered) and further investigated in Chapter 10. Sex ratios were investigated per site to look for any variations in populations visiting sites but are not investigated further in this section.

Site	Male count	% male	Female count	% female	Total
Sunlight	11	29.7	26	70.23	37
Lankan	144	36.5	250	63.5	394
Boduhithi	105	44.1	128	54.9	233
Table thila	180	37.3	303	62.7	483
Kalhahandi	9	32.1	19	67.9	28
Madivaru	48	27.4	127	72.6	175
Total	**497**	**36.8**	**853**	**63.2**	**1350**

Table 4.3 Sex of mantas (where recorded) identified at main sites.

4.4.3 *Review of size reported from observations*

Only 1382 records were investigated as the remainder had no record of size (DW). Seven *Manta birostris* are included in the Figures in Table 4.4 (Figures in brackets) but excluded in the analysis of size for *M. alfredi*. The maximum size increase observed was for a male manta that appeared to increase by 1 m disc width in three years (from 2.25 to 3.25 m DW). The majority of re-sighted animals did not increase in disc width or increased by 0.5 m or less.

Size (DW)/m	Females (n)	Males (n)	Total
1.75	1	1	2
2.0	18	72	90
2.25	0	2	2
2.5	113	188	301
2.75	3	25	28
3.0	299	188	487
3.25	6	6	12
3.5	259	18	277
3.6 (measured)	1		1
3.75	11		11
3.8 (measured)	1		1
4.0	149 (2)	5 (2)	154 (4)
4.1 (measured)	1		1
4.25	1		1
4.5	7		7
5.0	(1)	(1)	(2)
5.5		(1)	(1)
Totals	**873**	**509**	**1382**

Table 4.4 Number of mantas with estimated or measured size. Figures in brackets are *M. birostris.*

The *M. alfredi* size data was investigated using Minitab to determine whether there was a difference in size between males and females observed and to obtain a mean, median and maximum size for *M. alfredi* observed in the Maldives. Ranges of sizes of M. alfredi (Table 4.5) were 1.75-4.5 m DW for females and 1.75-4.0 m DW for males. The mean size (males and females combined) was 3.05 m (95% CI 3.02-3.08 m) DW and median size 3.00 m DW. This suggests that *Manta alfredi* in the Maldives is commonly sighted at 3.0 m DW and reaches a maximum size of 4.5 m DW. Female *M. alfredi* sighted were significantly larger than males (two sample t-test t = -22.89, $P < 0.001$) (Figure 4.44).

Variable	N	Mean (m)	SD (m)	Minimum (m)	Median (m)	Maximum (m)
All	1375	3.05	0.551	1.75	3.00	4.5
Females	870	3.26	0.513	1.75	3.25	4.5
Males	505	2.69	0.407	1.75	2.50	4.0

Table 4.5 Descriptive statistics: size (DW) of *Manta alfredi* in metres.

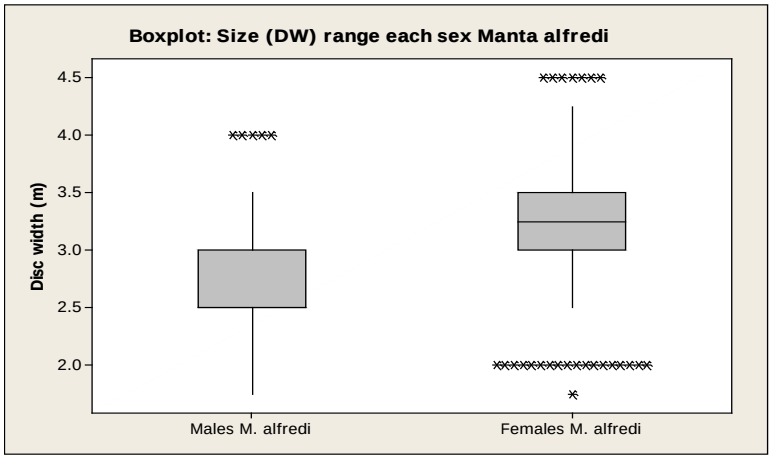


Figure 4.42 Boxplot graph of range of sizes of males and females (*Manta alfredi*). Females are significantly larger than males and the female range includes many smaller and larger outliers.

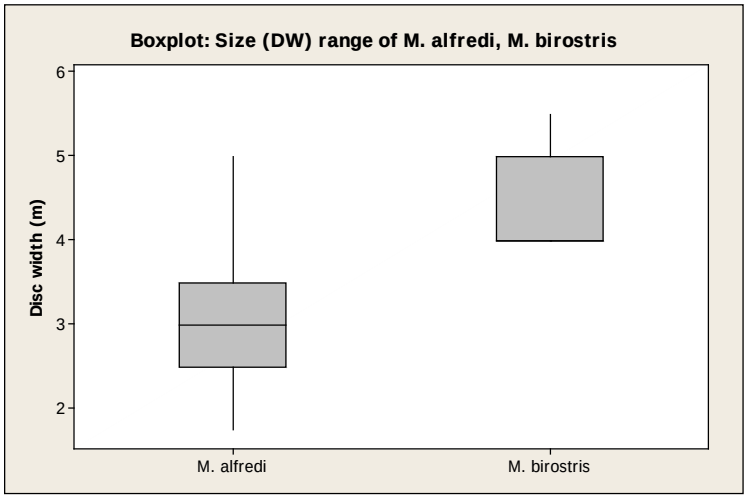


Figure 4.43 Boxplot graph of range of sizes for *M. alfredi* and *M. birostris* (sexes combined). *M. birostris* sighted in the Maldives are likely to be significantly larger than *M. alfredi.*

The range of sizes of the *M. birostris* sample were 4.0-5.5 m DW (the sexes were combined as the sample was very small n=7). The mean size of *M. birostris* sighted was 4.50 m (SD ± 0.645). It was known that *M. birostris* grows to a greater size than *M. alfredi* (Marshall at al. 2009) so this hypothesis was tested for mantas sighted from the Maldives. A two sample *t*-test was conducted to compare mean sizes (DW) of *M. alfredi* and *M. birostris*: $t = -5.94$, $P = 0.001$, DF = 6. *M. birostris* observed in the Maldives are likely to be significantly larger than *M. alfredi.* This result is shown graphically in Figure 4.43.

4.4.4 *Review of tail length reported from observations*

Tail lengths were investigated to determine whether there was a difference in tail length between sexes and if there was a correlation between tail length and size (DW). In the database, tail lengths were categorised as 'long intact', 'long cut', 'medium' and 'short'. Results are summarised in Table 4.6. Excluding unknown length, the modal length is 'long cut' for the entire sample but for females it is 'medium'.

Tail length/Sex	Female	Male	Unknown	Total
Short	228	72	1	301
Medium	274	104	2	380
Long Cut	227	192	0	419
Long Intact	156	134	8	298
Unknown	23	12	7	42
Total	908	514	18	1440

Table 4.6 Tail length count.

The relationship between size (DW) of manta and tail length (four non-parametric data sets) was first investigated to see if there was a significant trend using regression. A random sample of data from 500 mantas was analysed where the tail length and size (DW) were recorded. Tail lengths were allocated a value based on the approximate proportion of tail (criteria 3): Long Intact=1, Long Cut= 0.75, Medium =0.5, Short = 0.25. A weak negative correlation was obtained (S= 0.249, R^2= 8.3%), with regression equation Tail length= 1.06-0.141 size. The same data set was also analysed using Kruskal-Wallis test. The means ranks for the four tail lengths were significantly different ($H = 44.1$, DF = 3, $P < 0.001$ when adjusted for ties) and ordered from shortest to longest, supporting the hypothesis that tails length shortens with DW. A Tukey-Kramer all pair-wise comparison test indicated that the mean DW for all tail-length

pairs except short/medium and long-cut/long-intact were significantly different (P <0.05). The differences in mean DW were greatest between pairs with widest differences in tail length, providing additional support to the hypothesis that larger animals are likely to have shorter tails.

4.4.5 *Scars and missing tissue*

Based on records in the Manta details section of the Access database, 5% of recorded mantas bore evidence of attack or trauma resulting in loss of tissue or prominent scarring compared to 75% of mantas reported in Mozambique by Marshall and Bennett (2010a). Scarring may accidentally not have been reported if the scar/missing tissue (S/MT) was not observed during data collection nor captured on the photograph or video sequence used to identify the manta although careful review of the photo database supports the 5% result. There were only 10 manta records which contained a series of photographs showing S/MT over a period of years. This was due to the relatively small number of mantas bearing S/MT, the low-re-sighting rate and photographs missing the sections where the S/MT were likely to be located. In all cases where the series of photographs for an individual manta captured the area where S/MT were present, they were still evident in subsequent photographs; although some minor healing appeared to have occurred in some cases. There was no damage which had completely healed leaving no evidence on any of the 10 mantas. Notches and semi-circular areas of missing tissue showed least healing. An example is shown in Figure 4.46.

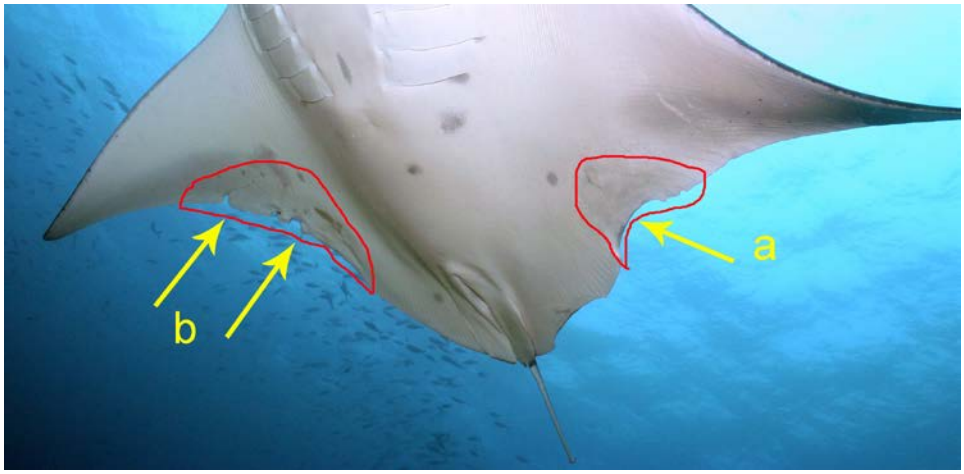


Figure 4.44 Photographs of scars and areas of missing tissue on 247 L222 taken on 11.11.2010 85 months after first sighting with these scar/missing tissue. (a) On the left wing (posterior) is a quarter-moon shaped area of missing tissue (likely, from a shark-bite). Around the bite area is a much larger area of thin tissue (ringed in red). (b) On the right wing (posterior) are at least two small notches and the area surrounding the notches (ringed in red) is thin.

This manta (ID number 247 L222 “Lucy”) was first reported on 24.10.2003 bearing a shark-bite (left wing, posterior), several notches (right wing posterior), and scars and thinned skin bilaterally around the area where the bite and notches were present. It is unknown what caused the areas of thinned skin. The manta was reported 10 times up to 11.11.2010 with the damaged areas healing, but still clearly evident. The photograph in Figure 4.46 was taken on the 11.11.2010, 85 months after the first report of this manta with the same damaged areas. For the 10 mantas with records of S/MT, the period between first and last record with the scars being evident ranged from 10 to 85 months. The mean time was 37.6 ± 19.7 months 95 % confidence interval, (SE 8.69 months, Anderson-Darling test for normality AD= 0.48, *P*= 0.179). From this result it could be concluded that missing tissue and scars would still evident for at least 18 months from first sighting in *M. alfredi.*

4.4.6 *Colour morphs*

1433 mantas (99.51%) were recorded as chevron *Manta alfredi.* 5 (0.35%) were chevron *M. birostris,* and 2 (0.14%) were black *M. birostris.* No black *M. alfredi* were reported. All black and chevron *M. birostris* were sighted only once. The first ever record of a black manta in the Maldives was of a 4 m DW male *M. birostris* from Kalhahandi reef in central west Ari atoll on 6.3.2003 (ID number 1001 K2). The first chevron *M. birostris* was also recorded on 6.3.2003 from Kalhahandi (ID number 992 K3).

4.5 Discussion

This is the largest database of mantas in the world and continues to grow by approximately 200 new mantas each year. Although the majority of mantas (75.55%) have only been recorded once, this method was useful in re-identifying individual mantas for the entire period of the study (c. 10 years). During this study evidence was published showing that mantas were born with a full set of markings (Marshall et al. 2008; Matsumoto and Uchida 2008). These observations, along with the report that manta 209 L181 was recognisable from a photograph taken in 1986 (G. Stevens pers. comm.), suggest that the ventral markings change insignificantly over the period of the animal’s lifetime. The method identified all mantas encountered when a good record was taken (i.e. sex, size, tail length and a good ventral photographs or video records were obtained). An all-white or all-black manta would not be individually identifiable by this method, but to date none have been reported. The markings are so individual that

each entry is unique. As described in the method, the occurrence of a single oval mark parallel to the fourth and fifth gill-slits in area 1 was relatively common (shared by 156 mantas, according to table 4.1). However, when the position of the oval and patterns of spots in Area 3 are also taken into account (when the final photo data base is checked), a match or the identification of a new manta is assured. The uniqueness of the ventral markings was recently corroborated by other manta researchers (Couturier et al. 2011; Deakos et al. 2011; Marshall et al. 2011).

Manta birostris vs. *Manta alfredi*

Manta birostris diagnostic characters: adult disc width (DW) exceeding 5 m (common) and to 6.8 m maximum, enlarged dermal denticles in upper jaw, presence of postdorsal protuberance with reduced caudal spine. Ventral markings patterns include no oval marks between gill-slits (area should be white, whether chevron on black colour type) but posterior wing margin pigmentation must be present which may include areas consisting of dark ovals which may be overlaid to create areas of block colour. Oval marks may also be located in area immediately posterior to gill-slits (central abdominal area). Oval marks located between gill-slits diagnostic for *M. alfredi. M. alfredi* (and not *M. birostris)* will also have random spot (not ovals) and/or mottled pigmentation in posterior ventral area.

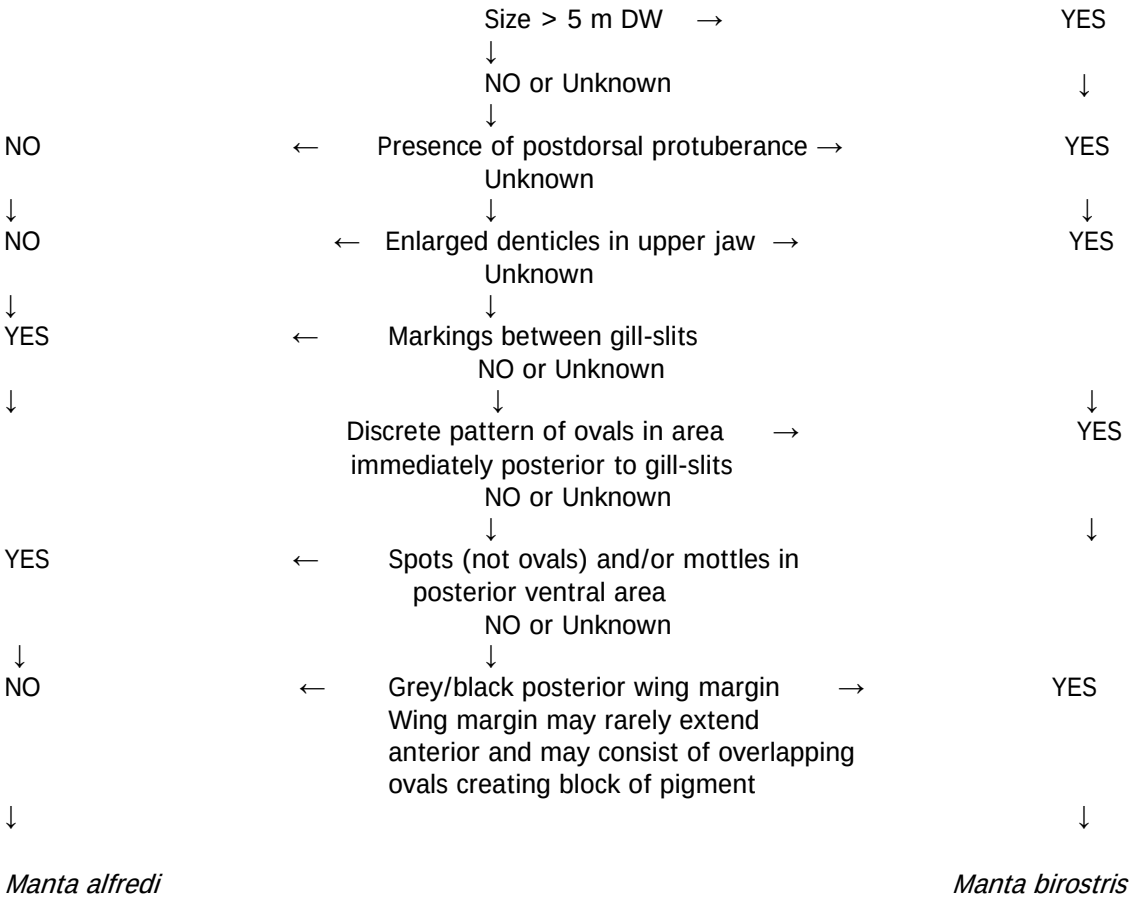


Figure 4.45 Species decision tree to identify *Manta birostris* from *Manta alfredi* from physical characteristics and markings, which may be reviewed by observation of mantas in the wild review, of dead samples or analysis of photographs. Once a query has been responded to by placing in extreme left or right column, follow to species name.

The method is useful for identifying differences between *M. alfredi* and *M. birostris* so that it is possible to identify the species from visual evaluation of photographs, video records and observation in the wild as was also suggested by Marshall et al. (2009) but no method given. In the Maldives, it appears that size can be a simple method to identify species when mantas are over 5m DW. Using the differences in markings patterns identified in this study, along with the physical differences (including enlarged dermal denticles in the upper jaw which are difficult to observe in the field and the presence of a postdorsal protuberance in *M. birostris* (Figure 4.46)) identified by Marshall et al. (2009), the species can be separated as summarised in Figure 4.45. Based on this study, the Maldives manta population is primarily (>99.5%) comprised of standard chevron *Manta alfredi.*

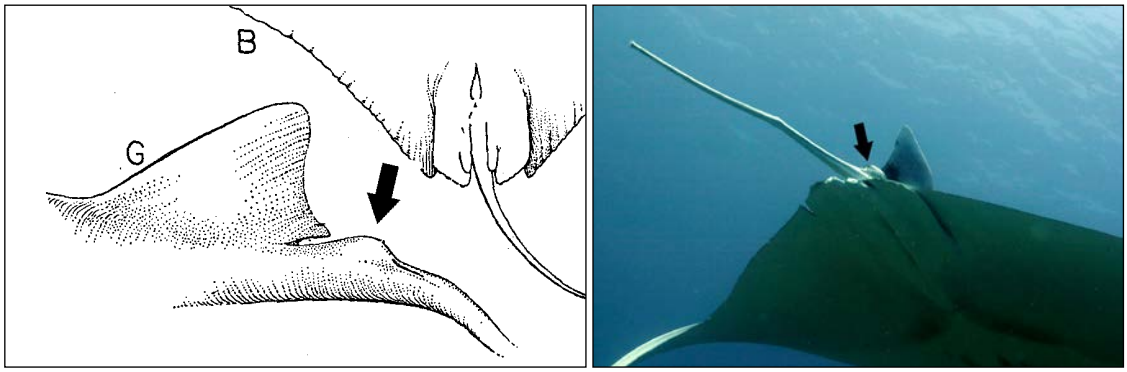


Figure 4.46 A postdorsal protuberance (PDP) is found in *Manta birostris* but not *M. alfredi* (Marshall et al. 2009). The drawing on the left from Bigelow and Schroeder (1953) clearly shows a PDP (marked with an arrow) from a 4.57 m DW female manta (location unknown) which was noted by the authors but was not identified as a potential differentiator of species. The photograph on the right was taken at Table Thila, Ari Atoll, Maldives on 25.3.2009 from a male manta with an estimated DW of 5 m.

A 1:1 primary sex ratio (at conception) would be expected in animals using sexual reproduction (Fisher 1930; Charnov 1982; Edwards 1998). In this study a strong female bias in the ratio of females to males was observed (1.8:1). The Marshall and Bennett (2010b) study in Mozambique showed a stronger female bias (3:1) whilst Deakos (2010) showed no significant bias (1:1.17) in the Maui, Hawaii population. Studies of shovelnose guitarfish *Rhinobatos productos* Ayres, 1854 and banded guitarfish *Zapteryx exacterata* (Jordan & Gilbert, 1880) showed the sex ratio of embryos to be 1:1 (Marquez-Farias 2007; Blanco-Parra et al. 2009). Many studies of wild elasmobranchs including diamond stingray *Dasyatis dipterura* (Jordan & Gilbert, 1880), sicklefin lemon shark *Negaprion acuditens* (Rüppell, 1837) and deepwater lantern sharks

Etmopterus spinax (Linnaeus, 1758) showed bias to one sex or the other with females being predominantly sighted or captured (Smith et al. 2007; Buray et al. 2009; Coelho and Erzini 2010). There is only one report of bias to the male sex where a ratio of 1:1.8 females to males was observed in blue shark *Prionace glauca* (Linnaeus, 1758) by-catch in the Mediterranean long-line industry (Megalofonou et al. 2009). A report of the sex ratios of 12 species of elasmobranch by Pikitch et al. (2005) suggested unity. However, variations in ratios were seen during annual cycles. No explanation of sex bias is given in any of these studies except in Marshall and Bennett (2010b) in which it was proposed that females congregated as a reproductive strategy with the study area being both a breeding and birthing ground. A possible explanation for the bias observed in the Maldives is that females are more likely to be seen at cleaning stations (where most surveys were conducted) due to their greater size and/or age. Older animals would require more cleaning maintenance and larger animals take precedence at cleaning stations (Bshary and Grutter 2002; Grutter et al. 2005). This hypothesis is investigated in Chapter 7.

Female *M. alfredi* were, on average, significantly larger than males in the Maldives however, the range of sizes for males and females were similar. Thus, some males may achieve the same size as large females. All of the largest mantas recorded were *M. birostris* ($\geq$ 5.0 DW). The largest *M. alfredi* recorded were 7 females of estimated DW 4.5 m which is within the range reported by Marshall et al. (2008) for this species with the largest reported worldwide at 4.9 m (White et al. 2006). These results are in agreement with other records of known *M. alfredi* populations (Myers 1999; Anderson 2005; Acker 2006; Laros 2006) that a typical size is 3.0 m DW. Mantas smaller than 1.75 m were not recorded from cleaning stations (see Chapters 7 and 10 for further investigations on size of mantas observed) but mantas as small as 0.9 m DW have been sighted in sheltered lagoons (for example Veyofushi lagoon in Baa atoll) but were not recorded (and thus missing from analyses) as not positively identified.

It appears that there is an inverse relationship of DW and tail length. Shark attacks on mantas were investigated by Marshall and Bennett (2010a). They reported that the majority of damage was made to the posterior edges of the pectoral and pelvic fins. It is likely that the manta was fleeing from an attack when the shark struck for the posterior area to have been bitten, and I speculate that the tail is probably the area most likely to be bitten on a shark attack and this would explain why the majority of mantas (78.7%)

observed in the Maldives have a shortened tail. As the number of shark attacks on a specific manta would be likely to increase with age, it would be expected that larger mantas could be expected to have a shortened tail. Oliver et al. (2011) reported the interactions between pelagic thresher sharks *Alopias pelagicus* Nakamura, 1935 and cleaner wrasse at sea mounts. They reported that 23.3% of inspections by cleaner fish were of the caudal fin area. Although a quantitative study of the number of cleaner fish cleaning specific areas of mantas was not undertaken in this study, it is very common to see cleaner fish around the end of the tail (e.g. Figure 7.6). I propose that the tail is likely to be further shortened by the actions of cleaner wrasses removing damaged tissue during visits to cleaning stations.

Buray et al. (2009) reported that healing of elasmobranch wounds including notches and splits, mating scars, and scars from nets and boat accidents healed within one year and could only be used for matching during a short period of time. Whilst some healing of scars and cuts has been observed in mantas, results from this study show that they would still be evident for at least 18 months after first sighting and that larger areas of missing tissue from shark bites (Figures 4.31 and 4.44) do not appear to heal substantially within 7-8 years based on repeat sightings of mantas 540 M42 and 247-L222 as missing tissue and scarring was still evident after that time. Using scars and missing tissue should not be used in isolation to identify mantas but provide corroborative support for positive identification.

The formal methodology proposed here is the first for visual identification of *Manta alfredi*, and whilst specifically designed to identify individuals within the Maldives population, can be used to identify individuals from other populations with similar ventral pigmentation patterns, for example, those in Hawaii, Yap, Bora Bora, Mozambique and Australia. The method can be adapted to include black specimens of *M. alfredi* and chevron and black *M. birostris* individuals which appear to have sufficiently different markings to enable identification of species. The report based on this chapter (Kitchen-Wheeler 2010) was the first record of black mantas and sympatry of species in the Maldives.

Chapter 5. Movements and migration of Alfred mantas (*Manta alfredi*) in the Maldives

5.1 Abstract

The recent separation of the genus *Manta* into two or more species has important implications for both the study of migration behaviour of manta rays, and their conservation. Worldwide, populations appear to exhibit three types of migratory behaviour which can be categorised according individual home ranges: resident, migratory, and oceanic. Resident and migratory behaviours describe activity by the smaller Alfred manta *Manta alfredi* whilst oceanic describes that of the giant manta *Manta birostris*, individuals of which are less commonly re-sighted and known to visit locations hundreds of kilometres apart. Using a visual identification method to record 2680 sighting of 1421 manta rays over a 7 year period at different cleaning station sites throughout the Maldives, individuals of *M. alfredi* have been shown to migrate annually between the western and eastern sides of atolls with the changes in monsoons, apparently benefitting from enhanced productivity on the lee-sides of the atolls. Pursuit of food was not the only factor involved in movements. Some animals made journeys between different atolls requiring the traverse of deep water (>600 m). Others visited different sites along the lee side of an atoll, and the chain of atolls, during a single season. The greatest distance between sightings for an individual manta was 270 km. This highly mobile behaviour has conservation considerations where protected communities are bounded by active fisheries.

5.2 Introduction

The recent recognition that the genus *Manta* contains at least two distinct species: *Manta birostris* and *M. alfredi* (Marshall et al. 2009) has important implications for the study of the migration behaviour of mantas. *M. birostris* occupies a wider range, is reported in both tropical and temperate waters, and known to travel distances of up to 613 km (Bigelow and Schroeder 1953; Rubin et al. 2008; Marshall et al. 2009). This species is reported to occur seasonally at various locations, including New Zealand (Duffy and Abbott 2003), Brazil (Luiz et al. 2009), Madeira and the Canary Islands (P. Wirtz, pers. comm.), and Ecuador (M. Harding, pers. comm.) but re-sightings of individuals were infrequent. For example, during 30 years of investigation, Rubin et al. (2008) identified 321 *M. birostris* from the Revillagigedos islands, Mexico. Individual mantas identified at cleaning stations were either never seen again or only re-sighted

after periods of years. Overall, only 33% of individuals were re-sighted with up to 10 years between sightings (Karey Kumli, pers. comm.). Little is known of the movements of this species other than that individuals may travel long distances and do not appear to remain resident in one area as adults.

Reports of manta rays known to be *M. alfredi* from Yap, Hawaii, Mozambique, eastern and western Australia, Japan, Komodo (Indonesia), Bora Bora (French Polynesia) and the Maldives indicate that individuals may be sighted repeatedly over periods of days, months and even years, visiting the same cleaning and feeding areas, and suggesting resident-type behaviour (Homma et al. 1999; Yano et al. 1999a; De Rosemont 2008; Deakos et al. 2008; Dewar et al. 2008; McGregor et al. 2008; Marshall et al. 2009; Kitchen-Wheeler 2010; Couturier et al. 2011). However, individual *M. alfredi* from the Yaeyama islands, Japan, have been observed to travel up to 400 km between groups of islands, and individual *M. alfredi* in eastern Australia were reported at sites along the coastline up to 500 km apart (Kashiwagi et al. 2010; Couturier et al. 2011). These movements suggest that individual *M. alfredi* are capable of long-distance migration, similar to *M. birostris*. The reports from Komodo, eastern Australia, Yap and the Yaeyama islands indicated that although manta rays are encountered year round, there was some seasonality of sightings with individual manta rays moving between sites depending on the time of year (Homma et al. 1999; Dewar et al. 2008; Couturier et al. 2011; Kashiwagi et al. 2011).

A definition of migration in animals proposed by Kennedy (1985) and refined by Dingle and Drake (2007) is that of a persistent, straight, movement behaviour resulting in relocation of an individual on a greater scale (both spatially and temporally) than its normal daily activities, and is thus a relatively long-distance movement of individuals. Additionally, migration would usually occur on a seasonal basis with movement “to and fro” between two areas. The movement may lead to the redistribution of individuals within a population. The phenomenon is found in all major animal groups and the trigger may be local climate, local availability of food, the season of the year or from behavioural stimuli e.g. mating reasons. Attenborough (1991) also suggested that to be counted as a true migration, the movement of the animals should be an annual or seasonal occurrence.

Based on these definitions the populations described from Komodo, Mozambique and Hawaii might be considered resident as the populations may be found within their daily range throughout a year (Clark 2008; Dewar et al. 2008; Marshall et al. 2010; Deakos et al. 2011). The populations in eastern Australia and southern Japan appear to include a number of individuals which make long distance movements, and in the former might be considered predictable due to seasonal changes, whilst other individuals remain in the same area year round (Kashiwagi et al. 2008; Couturier et al. 2011). The latter examples appear to be examples of simultaneously resident and migratory populations.

Relatively little is known about the geographical ranges of individual mantas. *M. alfredi* is commonly called the inshore or reef manta ray (Deakos et al. 2008; Marshall and Bennett 2010b; Couturier et al. 2011), suggesting that it inhabits shallow reef areas, however little is known of the ecology of this species and this may be a misnomer. *M. alfredi* (and likely *M. birostris*) have low fecundity with adult females capable of producing a maximum of one pup per year, and apparently failing to become pregnant annually (Marshall and Bennett 2010b) (and investigated in this study and reported in Chapter 10). The species are thus highly susceptible to overfishing and it is imperative for their conservation that more is known of migration behaviour as in Indonesia, India and along the east African coast there are fisheries that target mantas and could deplete migratory populations (Notabartolo-Di-Sciara 1995; Homma et al. 1999; White et al. 2006). These fisheries supply the Chinese medicines market or meet local demand for fish protein. Mantas are particularly heavily exploited in the Philippines, Mexico, Mozambique, Madagascar, India, Sri Lanka, Brazil, Tanzania and Indonesia (Camhi et al. 1998; Alava et al. 2002; IUCN 2007), and are now categorised as vulnerable to extinction on the IUCN Red List (Camhi et al. 2009).

For both species of manta, the majority of reported sightings were of individuals visiting known cleaning stations where small wrasses and other fishes remove dead skin, scar tissue and parasites from the client. In areas where the two species are sympatric, both species may attend the same cleaning stations (Marshall et al. 2009; Kitchen-Wheeler 2010; Kashiwagi et al. 2011).

In the Maldives, Anderson et al. (2011) indicated that mantas migrated from side to side of the atoll chain in phase with the monsoons in order to take advantage of seasonal plankton blooms and that the mantas were predominantly found on the lee side of the atolls to the prevailing monsoon winds. Scuba diving guides, literature and websites

direct potential observers of mantas to the leeward sides of atolls in order to maximise manta watching opportunities. For example, during the NE monsoon Harwood and Bryning (1998) advise divers to visit *Boduhithi Thila*, *Madivaru* and *Kalhuhadhihuraa Faru* (Kalhahandi Huraa) which are located on the west sides of North Male and Ari atolls. “Fairy Tale Manta point” (Fairytale Reef) and *Lankanfinolhu Faru* (Lankan Reef) were recommended to be visited during the SW monsoon and are located on the east side of North Male atoll (Figure 5.1).

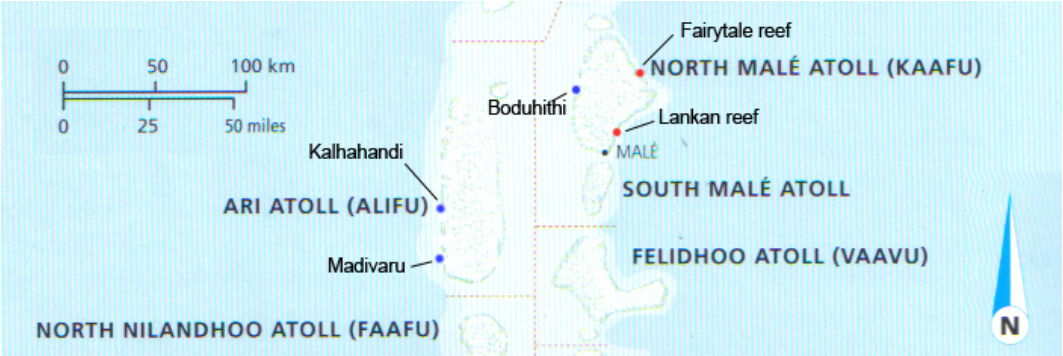


Figure 5.1 Diagram of central Maldives atolls from Harwood and Bryning (1998), indicating some locations where mantas may be observed by divers during the NE monsoon (blue spots) and the SW monsoon (red spots). The names of sites have been added. This map covers the area where the majority of surveys were conducted.

There appears to be a general agreement that mantas are seen on the seasonal leeward sides of the atolls. Once established that mantas are principally seen on the leeside of atolls, it is logical that there is a single population of mantas which moves from east to west (or vice-versa) with the change of seasons and not separate east and west populations (which do not visit the reefs for half the year since they are only seen on the leeward side). If there is a single population, the same mantas would be observed on both sides of the atolls during a year, but only on the side with the greatest plankton abundance in the appropriate monsoon.

This Chapter investigates the movements of *M. alfredi* in the Maldives as determined from repeated sightings of individual mantas which were individually identified using the method described in Chapter 4. The only previous scientific study of manta migration in the Maldives was the tagging project mentioned in Chapter 2 using colour coded “Floy” tags and only one tag was ever re-sighted (R.C. Anderson, pers. comm.). The ID method used here has been shown effective in recognising individuals over periods exceeding 7 years and can be used to monitor or detect movements of mantas

over periods of years rather than up to the ~12 months that have been achieved using acoustic tags and hydrophone arrays (Dewar et al. 2008) or satellite tags (Rubin et al. 2008).

This study aimed to identify patterns of movement by individual mantas to test the hypothesis that mantas migrate from one side of the atolls to the other with the seasons to take advantage of local plankton blooms and are thus generally seen on the leeward side of the atolls where plankton productivity is high (Anderson et al. 2011). Such seasonal movements would suggest migratory behaviour. The terms 'resident', 'migratory' and 'pelagic' or 'oceanic' are commonly used to describe the movements made by mantas (Last and Stevens 1994; Homma et al. 1999; Dewar et al. 2008) but they have not previously been defined. Based on the movements of individual animals in the Maldives, more precise definitions of these terms are suggested.

5.3 Method

To test the hypothesis based on the consensus of Anderson et al. (2011) and Harwood and Bryning (1998) that mantas are primarily seen on the leeside of atolls, it was necessary to analyse all sightings data and determine whether there was a relationship between the season and the location of survey sites where mantas are seen. The dates of all sightings were analysed to identify the season when the sightings were made and determine whether the sites would be considered leeward or windward at the time of each encounter. The null hypothesis would be that mantas are evenly distributed between atoll sides, irrespective of season. In practice, sites where mantas are not expected to be seen are less frequently visited, however Lankan reef in North Male atoll and Kalhahandi Huraa in Ari atoll, which are located on the outer reefs of the westerly and easterly margins (respectively) of their atolls, are visited by divers throughout the year and have adequate datasets (>5 surveys per year in both seasons over 5 years). Dive logs of visits to Lankan Reef and Kalhahandi Huraa were reviewed to determine whether mantas were seen and each survey date was allocated as windward or leeward depending on date. If mantas were seen at both sites year-round this would support the null hypothesis. The probability of reporting a manta from leeward and windward periods at each site was also determined. Additionally, all surveys conducted at dive sites where mantas are regularly sighted were investigated to determine whether mantas were reported or not and to identify which month and season the survey was conducted. The results can be used to account for bias in the survey rates between the leeward and windward sides in the analysis of probability of seeing a manta on either side.

Data from all surveys from November 2001 to November 2007 were analysed. The sightings data of over 1400 individual mantas were reviewed for date, time and location of each sighting. The survey data for sites where at least 10 surveys were conducted and mantas were reported at least once from the site are listed in Table 5.1. All surveys were conducted over approximately equal time intervals (45-60 minutes) so units of survey are comparable.

The number of surveys for each site when mantas were recorded was obtained from the Access database. This was to establish the probability of recording mantas at the main survey sites. A log of over 3000 dives made in the Maldives since 2001 was used to provide evidence of date, time and location of all surveys and used to determine whether mantas were seen or not and was particularly useful in providing data about manta presence (or absence) at windward sites, in particular Lankan Reef (North Male) and Kalhahandi Huraa (Ari). Throughout the period when manta surveys were conducted other dive sites were visited on both the leeward and windward, and inside the atolls for general tourist-diving purposes so if mantas were anywhere other than at the expected “manta points” they would be likely to be discovered.

The method of identification of individual mantas is described in Chapter 4 and the survey sites are described in Chapter 3.

The movements of individual mantas were investigated by analysing sightings data from North Male which has cleaning stations on both sides of the atoll that have been studied during both seasons. There were only data for sites on the west side of Ari atoll and east side for Baa atoll. Sightings from other atolls were too few for analysis. To ascertain whether individual mantas migrated between atoll sides, the Access database was queried for all individuals encountered at least twice and survey sites analysed for east and west atoll position.

By making a few assumptions, the frequency of visits to cleaning stations by mantas could be estimated from the re-sightings of individual animals at a particular cleaning station during a season, and the total survey time. If the total time that mantas were likely to utilise a cleaning station was calculated for a season, and the total survey time was known, then the observed visit time (for a specific manta, or an average manta etc.) can be multiplied up to estimate how often it would be expected to visit the cleaning station, assuming sampling is random. Lankan was the site with the most surveys (208)

and good success rate in recording mantas (during 85.1% surveys at least one manta was recorded (Table 5.1) so data from this site were used to investigate the re-sightings rate. Firstly, these data were analysed to predict the rate of visit by a 'typical' manta ray to Lankan cleaning station. To calculate the total time available for cleaning during the season, the following assumptions were made:

1. Surveys were primarily conducted in the SW monsoon during eight months from mid-April to mid-December thus sampling occurs over a period of approx. 243 days (8/12 x 365).
2. Manta rays visit Lankan cleaning station during daylight hours, typically from 06:30h to 17:30h, thus cleaning is likely to occur for a maximum of 11 hours in any day. Cleaner fish have never been observed to operate at night in Maldives (unpublished data).
3. Manta rays avoid the cleaning station at Lankan during periods of high current (Chapter 7). It was assumed that mantas were absent for 3 hours during the 12 hour daytime tidal cycle each day for the two weeks around new moon and full moon each lunar cycle when tides are greatest i.e. 2 out of 4 weeks, or half of the time available.

The sample of mantas used was the first 50 mantas reported from Lankan. This sample was therefore likely to be unbiased, and provided the maximum chance to be re-sighted during the 5 year period of surveys. The mean cleaning station visit time by manta rays at this location (35 minutes) was obtained by measuring the time spent by individual animals at the cleaning station during a full day (for some individuals this might be the cumulative time from several shorter visits during the day). See Chapter 7.

Sequences of individual manta sightings were also investigated to determine patterns of movement and distances covered during seasonal migrations and over longer periods of time (> 12 months). The distances of these movements can be estimated by plotting on a map. Case studies of such movements for individual mantas are described.

5.4 Results

Table 5.1 shows the number of surveys carried out per month over the period November 2001 to November 2007 at the survey sites where 10 or more surveys were conducted. The percentage of surveys when mantas were recorded ranged from 8.3% at Kani & Aquarium to 85.1% at Lankan Reef. Lankan was the most surveyed site because it offered the highest chance of a manta encounter. Other sites with greater than average chance of seeing mantas (percentage of surveys at that site when mantas were sighted

was greater than the mean of 64.8%) were Table Thila, Boduhithi and Madivaru. Generally, lower numbers of surveys were carried out during months when mantas were not anticipated.

Site	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Total A	No. surveys mantas seen B	% total
Sunlight	0	0	0	4	2	3	4	5	5	6	8	1	38	13	**34.2**
Lankan	6	4	3	5	20	13	60	25	19	25	18	10	208	177	**85.1**
Fairytale	0	0	0	0	1	2	1	0	3	6	2	0	15	4	**26.7**
Helengeli	0	0	1	2	2	1	2	0	2	2	2	1	15	2	**13.3**
Kani & Aq	0	0	1	3	1	1	1	1	3	1	0	0	12	1	**8.3**
Boduhithi	21	21	14	1	1	1	0	0	0	0	0	8	67	47	**70.2**
Table thila	11	13	18	5	0	1	3	3	0	1	0	9	64	52	**81.3**
Kalha-handi	11	11	12	6	0	0	6	5	1	3	1	11	67	14	**20.9**
Madivaru	21	23	20	5	0	0	1	1	0	1	5	25	102	71	**69.6**
Total	**70**	**72**	**69**	**31**	**27**	**22**	**78**	**40**	**33**	**45**	**36**	**65**	**588**	**381**	**64.8**

Table 5.1 Number of surveys conducted at each site per month from November 2001 to November 2007. The overall probability of recording mantas at each site expressed as a percentage is obtained from the number of times mantas were recorded at each site from the Access databases: B/A x 100%. Lankan site offers the best chance to see mantas during a survey.

1421 individual manta records from 2680 encounters where mantas were positively identified were reviewed for re-sightings. Of the 1421 mantas identified, 362 (25.5%) were re-sighted at least once (range 2-27 sightings) with the remaining 1059 (74.5%) seen once only. Sightings data for each survey site are summarised in Table 5.2. There is a strong correlation between percentage of mantas re-sighted and number of surveys conducted at the site (Pearson correlation r= 0.635, P = 0.008), indicating that with more surveys, the proportion of re-sightings will rise.

Using assumptions 2 and 3 listed in methods we may estimate that mantas may be present at Lankan cleaning station for, on average, 11- (3 x 14/28) = 9.5 hours each day. Therefore in one year (a season as mantas are absent during the opposing season) using assumption 1 there are 9.5 x 8/12 x 365 = 2312 hours when a manta ray might be at Lankan. There are no other known influences on the presence of mantas. Also assume that there is an equal chance of mantas being present during any hour in this time. The 177 surveys when mantas were reported is representative of the period of time when

mantas would be expected according to the assumptions. Each survey lasted ≈1 hour therefore each year we survey and see mantas at Lankan for an average of 35.4 hours which is only 1.53% of the available time.

Atoll	Survey site	Manta IDs (A)	Ever re-sighted (B)	% (C)	Multiple site (D)	East & West (E)	E & W as % of D (F)
N. Male	Lankan	408	166	40.7	85	82	96.5
	Boduhithi	237	52	21.9	22	22	100
	Kani & Aq	2	0	0	0	0	NA
	Sunlight	40	20	50.0	19	6	31.6
	Fairytale	8	2	25	1	0	0
	Total	**695**	**240**	**34.5**	**127**	**110**	**86.6**
Ari	Table Thila	486	73	15.0	6	3*	50.0
	Madivaru	182	47	25.8	9	1*	11.1
	Kalhahandi	38	2	5.3	1	0	0
	Rasdhoo	2	0	0	0	0	NA
	Ukulhas	1	0	0	0	0	NA
	Total	**709**	**122**	**17.2**	**16**	**4**	**25.0**
Baa	Nelivaru	2	0	0	0	0	NA
Raa	Kottefaru	3	0	0	0	0	NA
Nilandhe	Maavaru	2	0	0	0	0	NA
Addu	Mudakan	5	0	0	0	0	NA
S. Male	Guraidhoo	1	0	0	0	0	NA
Lhaviyani	Fushifaru	4	0	0	0	0	NA
	Grand total	**1421**	**362**	**25.5**	**143**	**114**	**79.7**

Table 5.2 Number of individual manta rays (A) known for each site, ever re-sighted, expressed as number (B) and percentage (C), number of individuals seen at more than one survey site (D), number seen on both sides of atolls (E) and mantas seen on both sides of atolls expressed as a percentage of mantas ever re-sighted (F). * Re-sightings were in different atoll as Ari atoll only has routinely surveyed cleaning stations on the west side.

To estimate the encounter rate over a 5-year period at Lankan, all sightings of a sample comprising the first 50 mantas encountered at the site (all first seen within 12 months of study start and within the first season of study at Lankan) were analysed. Over the 5 years, the mean encounter rate was 2.82 times (SD ± 2.83, range 1 to 12, mode/median=1), which equates to 0.56 times per year/season at Lankan only. Since

monitoring was carried out for only 1.53% of the total time that mantas would be expected to be making use of the cleaning station, we can estimate that a manta with the mean encounter rate would visit 0.56/0.0153 = 36.6 times each season which is approximately equivalent to a visit every 7 days. The most frequently sighted manta (and thus likely most frequent visitor) in this sample was sighted 12 times and would thus be estimated to visit 157 times in the season, which is approximately equivalent to two visits every three days. However, the median and modal encounter rate (once in 5-years) suggests that the majority of mantas visited 0.2/0.0153 = 13.1 times each season, approximating a visit every 19 days. See Chapter 7 for data and further analysis.

Whilst this results suggests variation in visitation behaviour by individual mantas to Lankan, it suggests that mantas may be cleaned daily (or at least, very frequently) as might be expected based on the being-cleaned behaviour of many fish species (Feder 1966). It was important to establish that mantas are cleaned frequently and would not avoid cleaning stations for six months of the year which might be suggested by them being absent from cleaning stations when they are windward. The behaviour of mantas whilst being-cleaned is investigated in Chapter 7.

5.4.1 *Testing of hypothesis: mantas are seen on leeward sides of atoll*

The season during which manta rays were seen at each cleaning station was ascertained. The results are summarised in Figure 5.2 and show that sightings on the western sides of atolls were mainly during the NE monsoon (sites marked by blue dots), whereas sightings on the eastern sides of atolls were mainly during the SW monsoon (marked by red dots). All survey sites from this study are included plus Muli (27) and Kurali (28) in Mulaku atoll and Maa Faru (16) in Baa atoll which are known manta points but there were no records in the database at the time of sampling (data of sightings of mantas at these locations came from other observers or from own log book records of mantas seen but not recorded). Additionally, many diving sites on both sides of and within the atolls were visited throughout both seasons so if mantas were present in an area they would be discovered (see discussion).

Mudakan (29) in Addu atoll and Kurali in southern Mulaku atoll are sites known for manta sightings in both seasons but have not been well studied. Apparently non-leeward sites where mantas were seen are Mulidhoo (1) and Desperation thila (2) in Haa Alifu, Rasdhoo North Channel (11) and Ukulhas (12) in North Ari atoll. All four of these sites

are located in areas of high plankton (assessed by horizontal and vertical water visibility and are within the plume shown in Figure 3.4 in Chapter 3) during the NE monsoon and are located on northern margins of atolls, unlike all remaining sites, so are neither truly windward nor leeward. This is discussed further in section 5.4.2. All four sites are considered NE monsoon sites (dive guide books recommend visiting them from December to April) and mantas have not been reported from these sites from visits and surveys made during the SW monsoon, so these sites are considered as leeside.

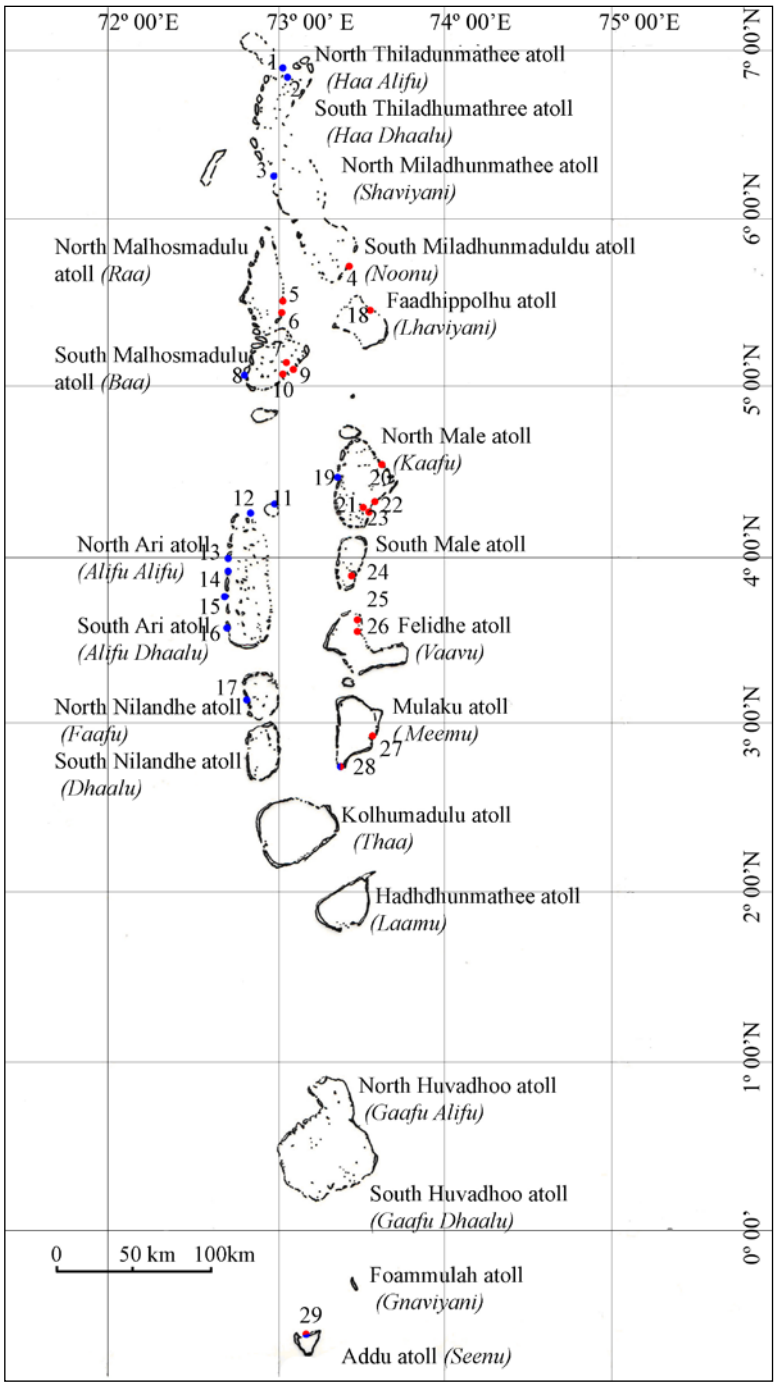


Figure 5.2 Maldives atolls with survey sites where mantas are regularly sighted marked. SW monsoon sites are marked in red, NE monsoon sites in blue. Site Key: 1 Mulidhoo corner, 2 Desperation thila, 3 Big thila, 4 Manadhoo, 5 Kottefaru thila, 6 Iguraidhoo thila, 7 Dhigu/Donfanu thilas, 8 Maa faru, 9 Hanifaru, 10 Nelivaru, 11 Rasdhoo North Channel, 12 Ukulhas, 13 Table thila, 14 Himendhoo/Emas thila, 15 Kalhahandi Huraa, 16 Madivaru, 17 Maavaru, 18 Fushifaru thila, 19 Boduhithi, 20 Fairytale reef, 21 Sunlight thila, 22 Kani/Aquarium, 23 Lankan reef, 24 Guraidhoo/Sandune, 25 Dhiggiri, 26 Alimathaa, 27 Muli, 28 Kurali, 29 Mudakan

To test whether there was a bias for sighting mantas on the leeward side, the two survey sites where surveys were carried out throughout the year were investigated (Lankan and Kalhahandi). Figure 5.3 shows box-plot graphs of the mean number of mantas seen per survey at both sites per month. The month data was obtained from pooling all surveys conducted during the relevant month from 2002 to 2007. At both sites there were periods of several months when no mantas were observed; these periods coincided with the time when the sites were windward. The number of mantas sighted during each survey ranged from 0 to 34 at Lankan and from 0 to 9 at Kalhahandi.

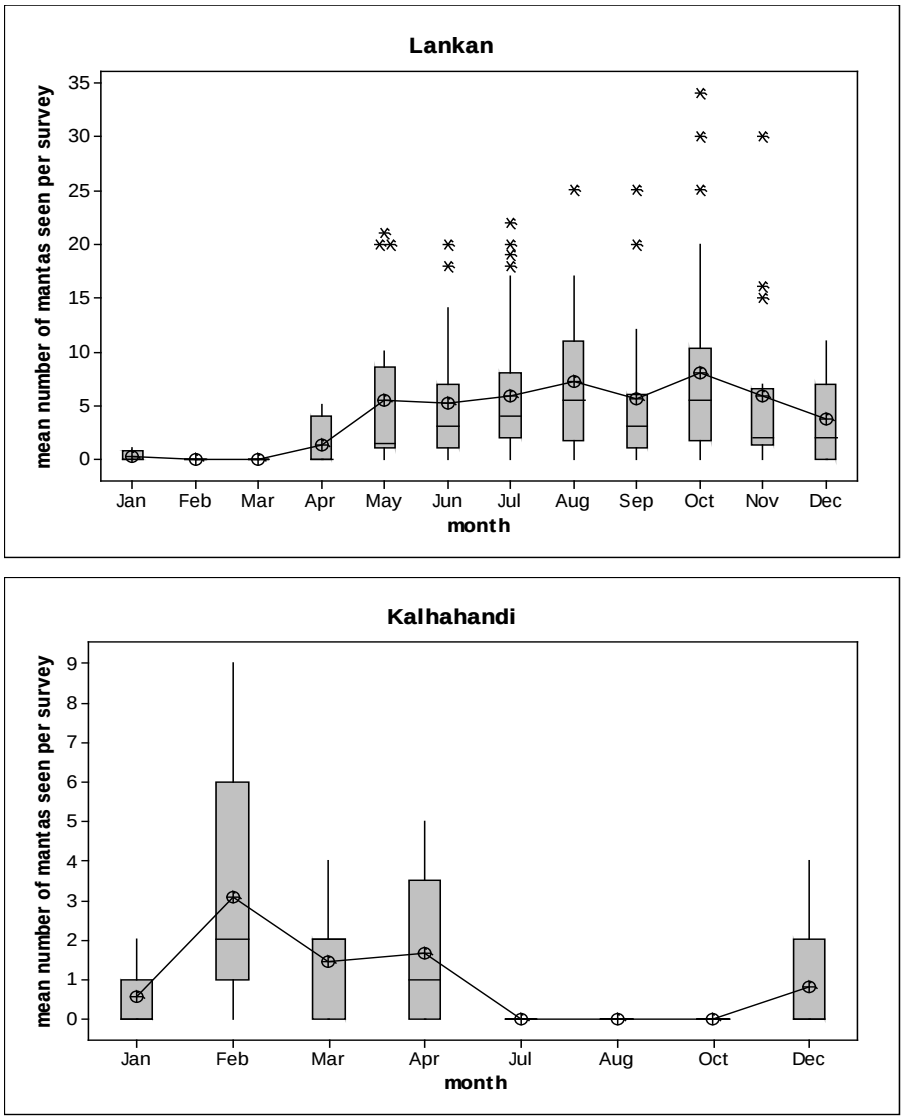


Figure 5.3 Boxplot graphs of mean numbers of mantas seen per survey each month at Lankan and Kalhahandi sites. Month data were pooled for surveys conducted 2002-2007. At Lankan few or nil mantas were sighted during January to March (the peak of the NE monsoon and at Kalhahandi none were observed from July to October (the peak of the SW monsoon). No surveys were carried out at Kalhahandi in May, June, September or November in any year due to high winds preventing access.

It is clear from the graphs that there were periods when nil or very few mantas were observed. These periods coincided with seasons when the sites were windward, so a Chi-square test was used to investigate these results. For the Chi-square test it was assumed that there was an equal chance of seeing mantas in either season. At Lankan during 21 surveys made during the windward period only one manta was ever seen. During 86 surveys made during the leeward period mantas were seen 73 times. Assuming that the chance of seeing mantas at Lankan should be the same in both seasons: $\chi^2 = 59.05$, DF = 1, $P < 0.001$. This result shows there was a significant bias to mantas being seen when Lankan was leeward. At Kalhahandi Huraa during 35 surveys made during the leeward period, mantas were seen on 22 occasions. During 15 surveys made during the windward period, no mantas were seen. Assuming that the chance of seeing mantas at Kalhahandi Huraa should be the same in both seasons: $\chi^2 = 24.8$, $P < 0.001$, DF = 1. This result also shows there was a significant bias to mantas only being seen when Kalhahandi was leeward.

Of all 2680 recorded manta encounters, only 6 (0.22%) (5 out of 592 encounters at Table Thila and 1 out of 1207 at Lankan) occurred on the windward side.

Surveys at all known sites where mantas may be seen were reviewed for the period between January 2004 and October 2007 inclusive, and surveys were categorised according to whether they were conducted during the leeward or windward season. The results are summarised as number of surveys per month in Figure 5.4. The lower number of surveys from mid-April to mid-December was because the SW monsoon is windier than the NE monsoon preventing surveys on windward sites. The allocation to monsoon for site varied from year to year for transition months due to weather variation.

Of 356 surveys analysed, 272 were on the leeward side and 84 on the windward side (3.23:1). This shows that although the majority of surveys were conducted when sites were leeward, a number of surveys were also conducted at sites when they were windward. The windward visited sites were: Lankan Reef, Boduhithi, Fairytale Reef, Helengeli Thila, Kani & Aquarium, Prisca, Sandune, Fushifaru Thila, Iguraidhoo, Kottefaru, Donfanu Thila, Dhigu Thila, Nelivaru, Rasdhoo, Rasdhoo North Channel, Ukulhas Thila, Table thila, Emas Thila, Kalhahandi Huraa, Madivaru, Maavaru and Alimathaa, Mudakan. These include the majority of study sites.

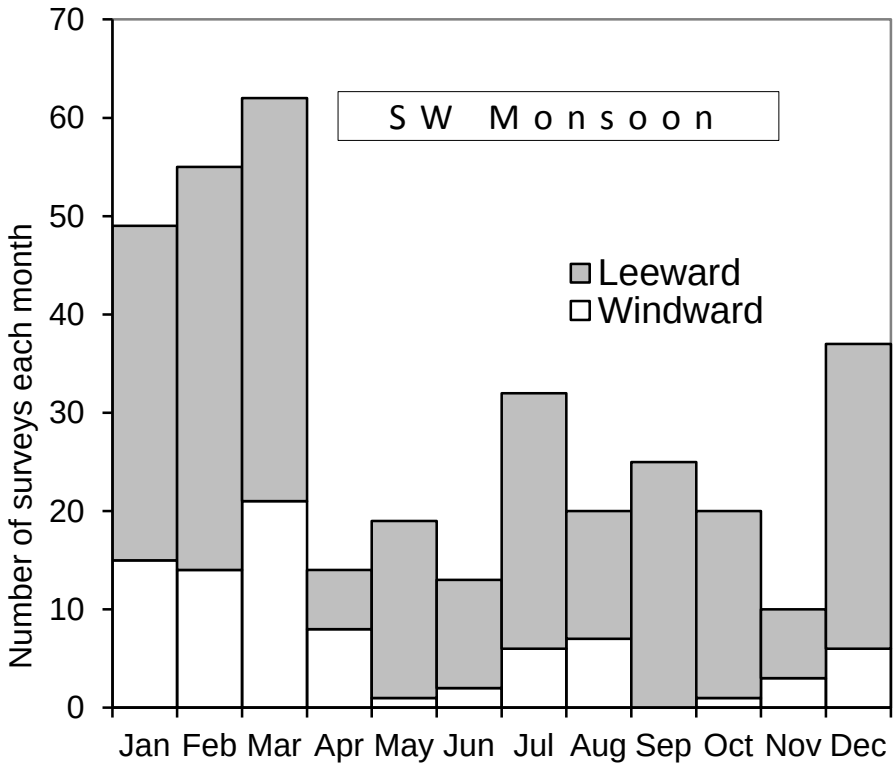


Figure 5.4 Distribution by month of surveys carried out between January 2004 and October 2007. Light-grey upper part of bar shows the number of leeward surveys and white lower part, number of windward surveys carried out at cleaning stations each month. Text SW monsoon indicates the period of the SW monsoon (mid-April to mid-November). Due to the calmer weather conditions during the NE monsoon, windward sites were surveyed noticeably more frequently during January to March.

Taking into the account the ratio of surveys between leeward and windward sites (leeward: 2674 manta encounters/272 surveys vs. windward: 6 encounters/84 surveys, thus survey ratio 3.23:1), if the same proportion of leeward and windward surveys were performed the encounter ratio of mantas leeward side to windward side would be 138:1 This result further supports the hypothesis that the majority of mantas are likely to be seen on the leeward side.

5.4.2. *Testing of hypothesis: the majority of mantas migrate from one side of an atoll to the other (with monsoon changes)*

127 manta rays in North Male atoll were observed at more than one site and 110/127 = 87% of these were observed on both sides of the atoll (Table 5.2). The animals sighted on both sides of the atoll were sighted an average of 6.0 times (SD ± 4.3, median 5 sightings). The remaining 130 (240-110) manta rays which were sighted more than once were seen on one side only of North Male atoll (at one or more sites). These animals

were sighted an average of 3.2 times (SD ± 2.3, median 2 sightings). Both data sets were non-normally distributed (AD =19.8, P <0.005; AD =5.8, P <0.005, respectively) so were compared using a Mann-Whitney U-test test. There is a statistically significant difference between the medians (U= 11665.0, P <0.05) rejecting the null hypothesis that the number of sightings of both groups of manta rays are the same. It would be expected that if manta rays cross from one side of an atoll to the other that the more times an animal was encountered, the greater the likelihood that it would be seen on two sides.

The most common pair of sites visited by manta rays seen on both sides of North Male atoll was Lankan Reef (east) and Boduhithi (west) accounting for 174 and 47 surveys, and 1154 and 556 encounters respectively (encounter rate per site 6.63 and 11.8 manta rays per survey, respectively). The other North Male sites (also on the east side) accounted for 18 surveys and 70 encounters between them. Boduhithi was the only west side survey site. So far, 110 manta rays have been sighted on both sides of the atoll, yet only 47/ (174+18+47) x 100 = 19.7% surveys were conducted on the west side. A discovery curve (Figure 5.5) was created to see how number of manta rays seen on both sides related to the number of surveys at Boduhithi (on the less-studied west side of North Male atoll). The linear regression forced through the origin indicates that for every 10 surveys at Boduhithi, on average another 27 manta rays are identified as having migrated from the better studied east side. If the number of surveys at Boduhithi were to equal the number on the east side (192), then the regression predicts that 518 manta rays would have been recorded from both sides of the atoll. The population study in Chapter 6 estimated the total population of manta rays in North Male to be 537 (SE±53.2). This result suggests the entire estimated population of manta rays in North Male atoll would have been seen on both sides if a sufficient number of surveys had been conducted on the less-visited west side. Importantly, this result suggests that it is not just a proportion of animals which migrate each season.

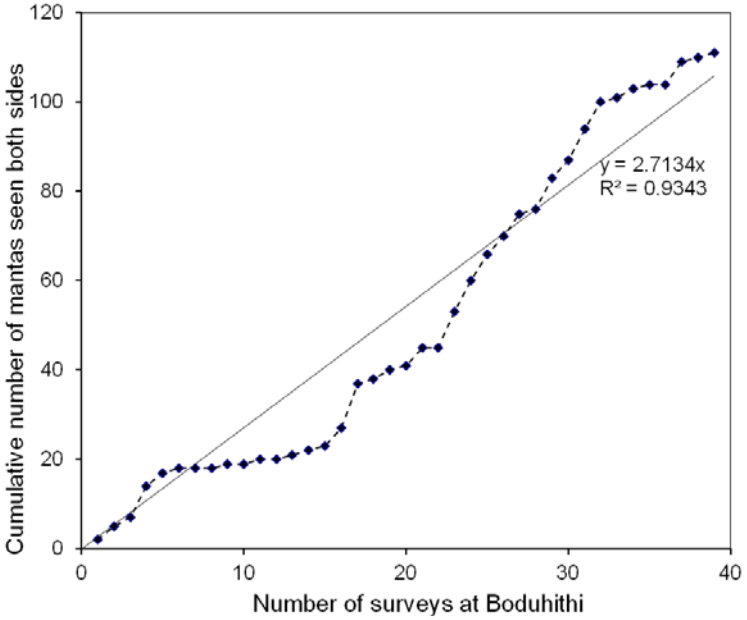


Figure 5.5 Discovery curve of cumulative number of manta rays seen at both sides of North Male atoll with number of surveys at Boduhithi. A linear regression line forced through the origin indicates that for every 10 surveys at the less visited site of Boduhithi, on average another 27 manta rays are identified as having migrated from the better studied east side.

No. sighted both sites/Distance (km)	Lan	Bod	Sun	Hel	Fair	Aqu	Madi	Tab	Kal	Maa	Fus	Nel	Desp
Lan		26.6	3.8	39.7	30.8	9.5	120	92		148		110	
Bod	110*		24.4		31.7			88			124	88	
Sun	25*	3											
Hel	1	0	0										
Fair	4	3*	0	0									
Aqu	1	0	0	0	0								
Madi	3	0	0	0	0	0		42.5	24.8	49.9			
Tab	5*	1*	0	0	0	0	9*		19.5	93.6			270
Kal	0	0	0	0	0	0	1	2*					
Maa	1	0	0	0	0	0	1*	1*	0				
Fus	0	1	0	0	0	0	0	0	0	0			
Nel	1	1	0	0	0	0	0	0	0	0	0		
Desp	0	0	0	0	0	0	0	1	0	0	0	0	

Table 5.3 Number of mantas seen at different site combinations/distance (km) for sites throughout the Maldives which mantas are known visit. Distances are not shown where no manta is known for the pairing. Lan=Lankan, Bod=Boduhithi, Sun=Sunlight, Hel=Helengeli, Fair=Fairytale, Aqu=Aquarium, Madi=Madivaru, Tab=Table thila, Kal=Kalhahandi, Maa=Maavaru, Fus=Fushifaru, Nel=Nelivaru, Desp=Desperation thila.* includes mantas seen at this pairing in combination with other site.

The number of manta rays recorded for each pair of sites is listed in Table 5.3. The most common pair of sites is Lankan and Boduhithi. The relationship between the distance between sites and the frequency of the pairing reported was investigated but there was no significant correlation (Pearson correlation -0.237, $P = 0.315$, Kendall tau (T) = -0.317, two-sided $P = 0.079$). The relationship between sites is also shown in Figure 5.6.

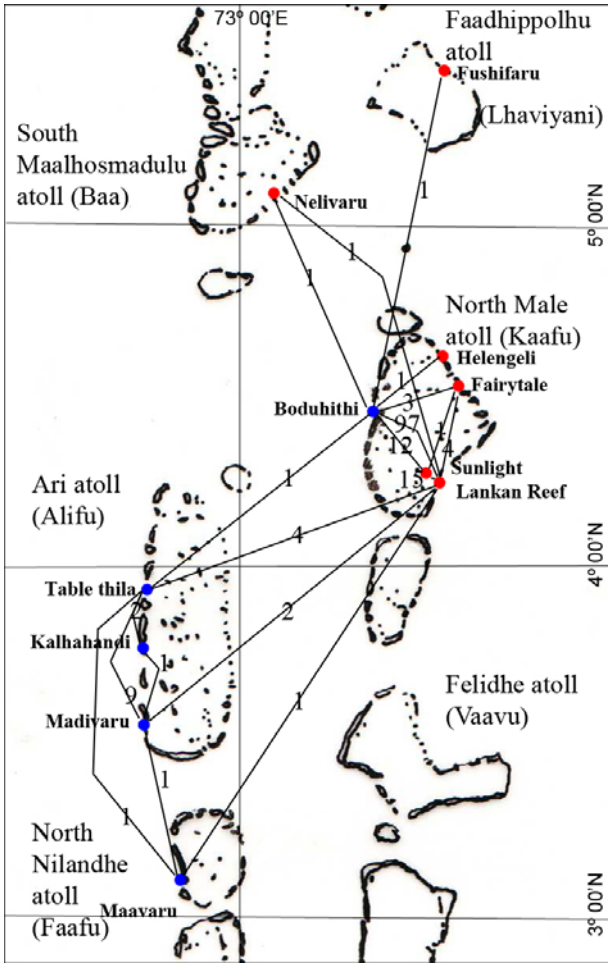


Figure 5.6 Diagram of central atolls with survey sites marked. Numbers of mantas seen at pairs of sites are shown on lines linking them.

5.4.3 *Case studies of individual manta movements to investigate migration routes and distances travelled*

Table 5.4 lists seven examples of migrations observed with a diagrammatical representation in Figure 5.7. The most common movement was as demonstrated by manta 243 L218 which was a movement across North Male atoll to visit cleaning stations on both sides of North Male atoll in the appropriate monsoon (110 manta rays were seen on both sides of North Male atoll).

Manta Ray ID	Description of migration/movements	Comments
243 L218 Large adult female	Migrates between Lankan (E side) and Boduhithi (W side) NMA. Only reported from these two sites (Figure 5.6 route 1)	Reported on both sides of atoll during appropriate season since 2003. These sightings suggest she makes annual migration across atoll and back with changes in monsoon as predicted by changes in productivity in atoll
434 L371 Adult female	Travels from Lankan (E side North Male atoll) to Nelivaru and Hanifaru (E side Baa atoll) in SW monsoon and also Boduhithi (west North Male atoll) in NE monsoon (Figure 5.6 route 2)	Travels long distances between sites on E sides of different atolls and to W side of NMA. This requires traverse of open ocean (> 600m depth) with shortest distance 90 km and then back to visit west side sites when leeside sites are available in both atolls from where she is known. The inter atoll movement appears unnecessary
184 L153 Sub adult when first sighted now adult	Initially sighted at Table Thila on west side AA in NE monsoon but since late 2003 sighted only in NMA at both Lankan (E side) and Boduhithi (W side) (Figure 5.6 route 3)	This manta appears to have migrated from the west side of AA to NNA during 2003 where she appears to have remained and now observed on the appropriate side of atoll with the relevant monsoon She is a known associate of 243 218 (above)
1108 D459 Adult male	Alternately sighted at Lankan (E side NMA) and Table Thila (W side AA) in appropriate opposite monsoons (Figure 5.6 route 4)	Travels between atolls to visit appropriate seasonal leeside cleaning stations.The distance between sites is at least 90 km and involves crossing deep water (300-600 m depth). This movement suggests there may not be appropriate SW monsoon cleaning stations on E side of AA
292 L269 Sub adult at all sightings	Sighted at Madivaru (W side AA) in NE monsoon, next sighted at Lankan (E side NMA) during SW monsoon (route 4) and then at Maavaru (W side NNA) during NE monsoon (Figure5.6 route 5)	Another manta reported travelling between W AA and E NMA but also travelling further south to NNA. This is a sub-adult so would not be looking for mates. The shortest distance between Madivaru to Lankan and Maavaru is 250 km
647 M159 Female Sub-adult at first now likely adult	Reported from Madivaru and Table thila (W side AA) and Maavaru (W side NNA) Figure 5.6, route 6). The west side of the channel between AA and NNA exceeds 100 m depth	Sightings records suggest she migrates up and down the west side of Ari/North Nilandhe atolls during the NE seasons but it is unknown where she goes during the SW monsoon season. This example shows that manta rays can use different cleaning stations up to 100 km apart (distance from Table thila to Maavaru) on the leeward side
823 D123 Sub-adult when first sighted, then adult	This ray was first seen at Table thila (W side AA) during NE monsoon 2003 and then in NE season of 2008 at Desperation thila in Haa Alifu atoll in the far north of the Maldives	She was not seen at Table thila after 2003 and her whereabouts in the interim are unknown. The shortest distance between these sites is 270 km (Figure 5.6, route 7)

Table 5.4 Examples of migrations determined from observations of individual mantas at two or more cleaning stations. NMA= North Male atoll, AA= Ari atoll, NNA=North Nilandhe atoll.

There were no cleaning stations on the east side of Ari atoll which were regularly visited by divers (although there are many diving points along the east side which are visited by divers throughout both seasons, but mantas are rarely sighted and none identified, so far). The only re-sightings of mantas in the SW monsoon which had been previously reported from Ari during the NE monsoon were at North Male atoll cleaning stations (1108 D459 and 292 L269).

At least one manta ray was recorded as migrating between Lankan (North Male atoll) and Table thila (Ari atoll) over several years supporting the hypothesis that there were not appropriate SW monsoon cleaning stations in Ari atoll. Five mantas were reported travelling between same-side sites in different atolls (the examples here are 434 L371 and 647 M159) indicating that individuals will travel long distances between different cleaning stations even during the same monsoon. The distance of 270 km between sites where 823 D123 was sighted is the largest reported one-way movement by a manta in the Maldives.

There were relatively few manta rays (11 out of 1421 = 0.77%) recorded moving between atolls, but this may be due to the low number of surveys outside of North Male and Ari atolls at the time of sampling of the database (November 2008). The database was re-sampled in November 2011 when around 20% of the surveys were performed outside of North Male and Ari atolls and only 20 out of 1947 (~1%) mantas rays were recorded moving between atolls. These results suggest that the level of movement between atolls is low.

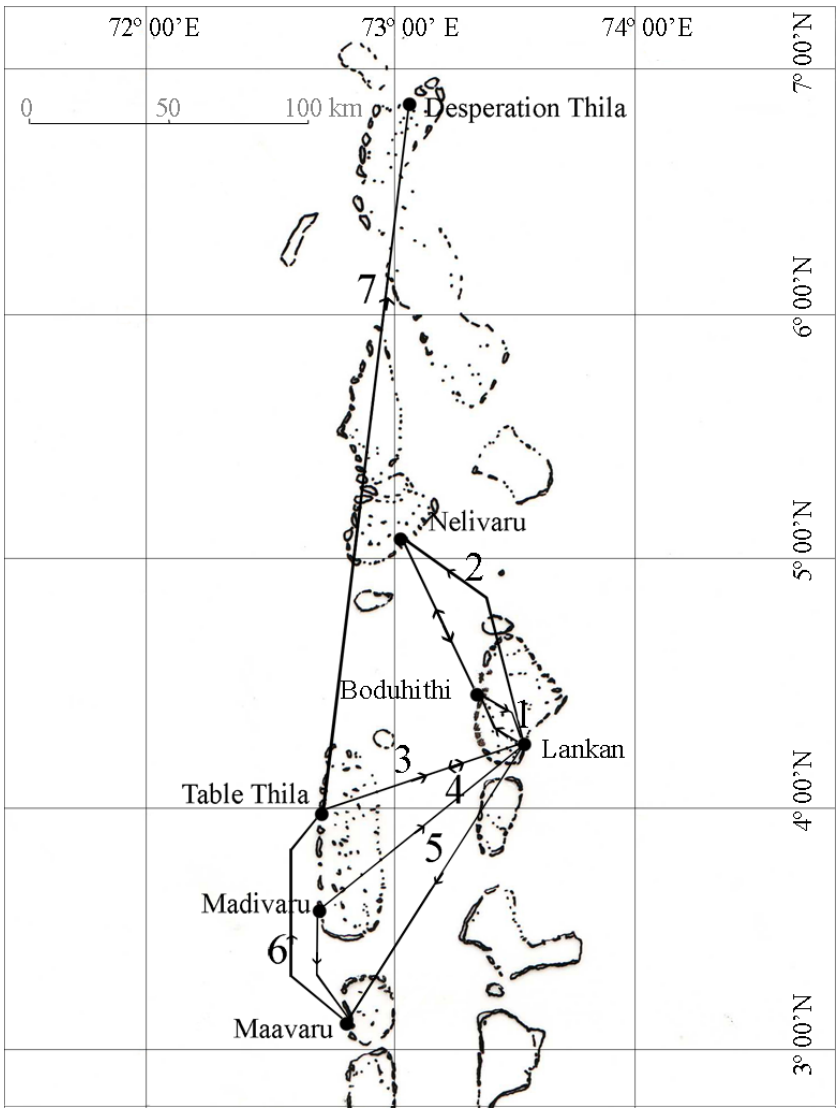


Figure 5.7 Seven types of movement shown by individual mantas. (1) Seasonal migration between Lankan and Boduhithi (the most common known annual migration) for more than 5 years by manta 243 L218. (2) Movement northwards to Nelivaru from Lankan in same season then between Boduhithi and Nelivaru by manta 434 L371. (3) Table Thila (Ari atoll) to Lankan and then seasonal migration between Lankan and Boduhithi by manta 184 L153. (4) Apparent seasonal migration between Table thila and Lankan by 1108 D459. (5) Long distance inter-atoll (Madivaru-Lankan-Maavaru) movement by 292 L269. (6) North and south inter-atoll movement by 647 M159. (7) Long distance (270km) one way movement by 823 D123 from Table to Desperation Thila.

5.5 Discussion

5.5.1 *Frequency of sightings and frequency of cleaning station visit by M. alfredi in the Maldives*

Only 25% of all individually identified mantas (1421) were sighted more than once. Even in North Male atoll where the highest proportion of surveys was carried out and sites are visited in both monsoons only 34.5% of mantas were re-sighted, although

mantas first reported at Lankan and Sunlight show a proportion of re-sightings closer to 50%. There is a strong correlation between percentage of mantas re-sighted and number of surveys conducted at the site (Pearson correlation= 0.635, *P*= 0.008), indicating that with more surveys, the proportion of re-sightings rises. It was expected that a low rate of re-sighting may have been due to a low rate of visits to cleaning stations by mantas, but analysis from the sample of 50 mantas first seen at Lankan suggests that an 'average' manta visits approximately every 7 days yet would only be re-sighted approximately every two years due to the low rate of survey. A frequent visitor, attending at Lankan approximately two out of three days, would only be seen 2-3 times during the season. The even lower survey rates at other sites would be expected to produce the lower re-sighting rates obtained.

Studies of *M. alfredi* in Maui (Deakos et al. 2008) had 72% of mantas re-sighted during a 4 year study of 247 individuals suggesting a similar pattern of infrequent sightings but the report did not include the data required to make a direct comparison on frequency of cleaning station visits by this population. The study on the behaviour of *M. alfredi* while being cleaned is reported in Chapter 7.

5.5.2 *Manta rays are seen on the leeside of atolls*

The review of sites where mantas are reported in the Maldives and summarised in Figure 5.2 indicates that the majority of sites are to leeward when mantas are sighted and provided initial support of the hypothesis that mantas are seen on the leeside of atolls. Exceptions were located on the north and south margins of atolls, but none were on the windward margin of the atolls chain when mantas are observed. The processed satellite images of chlorophyll-a concentration for the NE monsoon in Figure 3.3 show a plume extending from the east margin of the Maldives across most atolls. Knowing that mantas feed on zooplankton, which in turn feeds on phytoplankton supported by nutrient upwelling, we could reasonably expect mantas to be absent or rare from the extreme windward side of the Maldives which would have lower food availability.

Section 3.3 describes the effects of currents and tides on movement of plankton. Windward facing reefs on all atolls appear to experience an almost continual inward flow of water whilst leeward reefs are subject to inward and outward flows. Mantas exhibit diurnal changes in activity, visiting shallow reefs during daylight and travelling offshore at night to feed on the deep scattering layer, at up to 150 m depth (DSL)

(Robinson and Gomez-Guitierrez 1998) as alluded to by Dewar et al (2008) and reported by Clarke (2008) (mantas were reported in both studies as *Manta birostris* but are known to be *M. alfredi*). Mantas were often observed opportunistically feeding in shallow water during the day as reported by Dewar at al. (2008) and in the Maldives as reported in Chapter 8.

Water movement in the Maldives is notoriously hard to predict. It may be this complicated system involving wind, oceanic current, tidal flow and geographical factors (as discussed in Chapter 3) which cause unpredictable movement in certain channels resulting in high plankton concentration in, or nearby channels which might be expected to be partially windward e.g. Rasdhoo North Channel, Ukulhas and Mulidhoo. From regular observation, all three sites have <15 m horizontal visibility throughout the NE monsoon, but have much clearer water surrounding them during the SW monsoon. Investigating the mechanism by which these areas trap plankton is beyond this study but the observations of plankton enriched water surrounding these sites might explain why mantas might visit appropriate nearby cleaning stations to be cleaned. Desperation thila is neither windward nor leeward as it is located centrally, close to the northern edge of the atoll, a little south of Mulidhoo (10 km distance). The sites are close enough that mantas may forage (or be cleaned) at Mulidhoo and visit the thila within less than an hour's swim. Kurali is located in the southernmost channel of Mulaku atoll which apparently has water outflow almost year round and low visibility conditions. Mantas have been sighted there year round (Lisa Allison, pers. comm.). Mudakan site in Addu atoll has cleaning stations located inside the channel, within the atoll. Due to poor movement of water in and out of the atoll (partially caused by causeway construction along the entire SW atoll margin) visibility levels are much lower inside the channel (and within the atoll) than on the ocean facing reef and mantas are reported in both monsoons.

In summary, there are no sites where mantas are regularly seen when they are windward facing. They are almost always seen at leeward sites and the exceptions are located near the northern or southern margins of atolls that experience high plankton productivity during periods when mantas are seen. That 99.78% of manta encounters are on the leeward side vs. 0.28% on the windward side strongly supports the leeside hypothesis. When adjusted to take into account the bias towards leeward surveys the ratio of mantas seen was very strongly to the leeward side (138:1). The single sighting at Lankan from

the windward season was during a period of unusual conditions in early January 2005 when the normally 30 m+ visibility expected during the NE monsoon was replaced with low visibility (<10 m) from the presence of plankton (reason unknown). A juvenile whale shark was also observed feeding in the shallows during the same survey. The results from surveys from 2002 to 2005 at the two sites visited year round (Kalhahandi Huraa and Lankan Reef) were also supportive of the leeside hypothesis.

'Mantas are seen on the leeside' hypothesis needs clarification. It is better stated 'mantas are seen on leeside reefs when conditions become conducive to the presence of mantas due to high plankton productivity in nearby areas'. The important factor appears to be the presence of plankton. Although the majority of observations were of mantas being cleaned (the surveys were at cleaning station sites) it is probably the foraging opportunity which drives the migration in the Maldives, as proposed by Anderson et al. (2011). The presence of food nearby was suggested as the factor driving visitation to nearby cleaning stations by *M. alfredi* in Komodo and Hawaii, reported by Dewar (2008) and Clark (2008) respectively. In the Maldives, a change in wind direction for a few days will not affect the presence of mantas as it will not significantly affect nutrient upwelling and the local food chain.

5.5.3 *Individual manta rays travel from east to west sides of atolls with seasonal changes*

The analysis of data of re-sighted manta rays shows that a significant number of individual manta rays travel east to west, across an atoll and across the chains of atolls. Most evidence was from North Male atoll where surveys are carried out throughout the year. There is a large difference between the percentages of manta rays re-sighted in North Male (34.5%) compared to Ari (17.2%) despite a similar number of manta rays known from each atoll. This is explained by a greater number of surveys being carried out in North Male compared to Ari (241 vs. 142). In addition there are no known sites in east Ari where manta rays can be reliably seen so data captures are only performed for 4-5 months of each year during the NE monsoon.

In North Male atoll, that 87% of mantas which visited more than one site were reported from both sides of the atoll is strong evidence that mantas migrate with the seasons. Additionally, mantas which visited sites on both sides of the atoll had been sighted significantly more frequently than those which visited one side only as would be

expected: the more frequently a manta is sighted, the more likely it would be reported on both sides if they migrate. Of mantas seen relatively frequently (>6 sightings), the majority (79.2%) had been seen on both sides of the atoll. It is expected that with more surveys and more even sampling of east and west sites that the percentage seen on both sides would get closer to 100%. This is strong evidence that individual mantas make seasonal migrations from east to west and there are not separate east and west populations.

Of mantas seen at more than one site, the most common pairing of sites is Boduhithi and Lankan in North Male with 110 mantas being seen at both sites (70.78% mantas sighted at more than one site visited both these sites). This pairing of an east and west site is to be expected based on the hypothesis that rays migrate east to west in the appropriate monsoons. Lankan and Boduhithi are approximately 30 km apart (by the most direct route) and it is unknown whether they swim through, or around the atoll (a minimum distance 63 km). These distances are within the known capability of a single day swim as indicated by Dewar et al. (2008) but there is no evidence that they make daily swims between opposite sides, whilst there is evidence that they are absent from sites when they are windward. It is noted that the survey sites were primarily cleaning stations (not feeding areas) and the cleaner fish were observed to remain in attendance year round and clean other clients in the absence of manta rays. Thus, mantas could continue to use the windward cleaning stations but do not.

There were fewer data for Ari atoll and comparatively few mantas had been seen at more than one site. The most common pairing of sites in Ari atoll is Table thila with Madivaru (8 mantas seen at both). With no known regular SW monsoon survey site in Ari it is necessary to look beyond Ari for relationships between sites. It would be valuable to satellite tag some of the large mantas seen frequently at Madivaru and Table thila to discover where they migrate to in the SW monsoon. It is possible that there are several manta cleaning stations in Ari on the east side but they may be deep sites or away from tourist dive points. Four mantas were reported to have migrated between Table thila and Lankan, in North Male atoll, and one has now been seen several times alternately at both sites in the appropriate monsoon over a period of three years. This movement suggests a lack of suitable Ari SW monsoon cleaning stations so that Ari mantas need to travel to east North Male (or more distant atolls) in the SW monsoon. That manta rays move north and south along the ocean facing reefs of both Ari/North

Nilandhe and North Male to visit different cleanings stations (Table thila to Kalhahandi Huraa, also Madivaru to Maavaru, and Lankan to Fairytale etc.) during a single monsoon indicates they are highly mobile and individuals are not reliant on a single cleaning station. All inter-atoll movements require the traverse of moderately deep water (>300 m depth) with depths between some atolls exceeding 1500 m (Godfrey 2004).

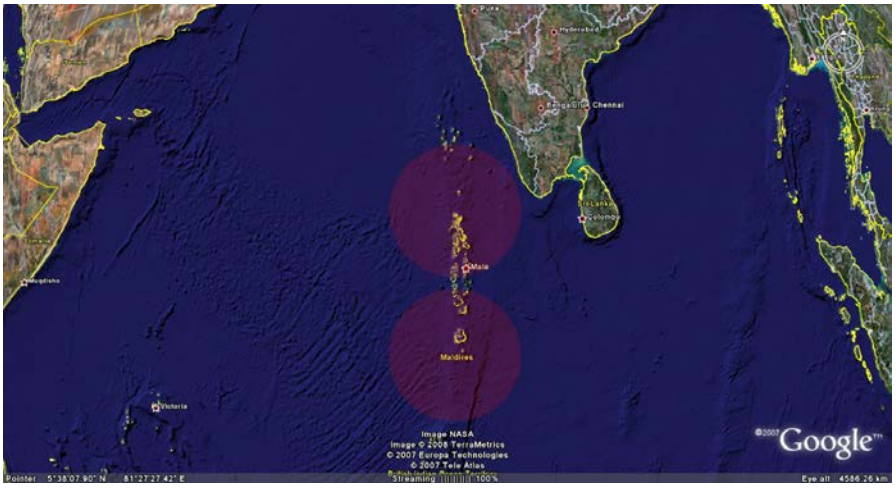


Figure 5.8 Google map of the Indian Ocean with the Maldives in the centre. The two circles indicate areas within a radius of 300 km from the extreme north and south of the Maldives and are indicative of distances individual *Manta alfredi* have been known to travel in the Maldives. The northern circle encompasses some of the Lakshadweep islands and comes close to the SW coast of India.

The distance travelled by manta ray 823 D123 in a single direction (270 km) has important ramifications as it confirms that Maldives *M. alfredi* may complete one-way movements of a few hundred kilometres and will cross deep ocean water (>600 m deep). There is a likelihood that they may migrate further north towards the Lakshadweep islands and the Indian mainland, possibly north-east to Sri Lanka and south to Chagos as indicated in Figure 5.8. These countries do not offer the protection from fishermen enjoyed by mantas in the Maldives.

5.5.4 *Definitions of types of ranges*

Telemetry/migration studies of mantas have been carried out in Yaeyama in Japan (Yano et al. 2000), Komodo Marine Park in Indonesia (Dewar et al. 2008) and Kona and Maui, Hawaii, USA (Clark 2008; Deakos et al. 2011). Dewar et al. (2008) used acoustic tags and hydrophones at eight locations around the marine park in Komodo to study the movement of 41 individual mantas (reported as *Manta birostris* when the

genus was considered monospecific, but clearly *M. alfredi* based on ventral markings). Mantas were present in the park year round even though some individuals were not detected for several months each year. Individuals that remained would typically visit the same sites throughout the year. A distinct seasonal pattern was identified in both north and south Komodo with mantas being more abundant in the north during the winter, and south during the summer, but mantas were seen year-round at north and south locations. Concentration of prey was proposed as a critical motivator for mantas being observed in a particular area. The area of the marine park is relatively small at 625 km² (compared to 2026 km² for North Male atoll) and hydrophones were spaced c. 20 km apart. Manta rays were primarily re-detected at the site of tagging although some movement occurred. These results suggest that the Komodo mantas were resident, visiting sites in the marine park year round and making daily visits to feeding areas and cleaning stations, travelling a distance of up to 40 km daily (round trip), and returning to the open seas at night. The authors also suggested that there might be 'residency patterns' within the park but these were not defined. Mantas which were not detected for months at a time may have simply been visiting another cleaning area without a hydrophone, have lost their tags, or have left the park.

Studies of *M. alfredi* in the Hawaiian Islands by Clark (2008) in Kona, Hawaii and Deakos et al. (2011) in Maui, showed no migration of animals between the islands (130 km apart) suggesting that individual mantas were 'resident' to an island. Telemetry studies and visual identification studies around Kona showed individual mantas to be reported year-round at the same survey sites (T. Clark, pers. comm.). Clark (2008) tracked 37 individual manta rays using active and passive tracking and passive receivers. Results showed mantas visiting reefs in the morning to visit cleaning stations and then travelling offshore in the afternoon and evening to feed, visiting deep water feeding sites after 03:00 hours. The greatest numbers of mantas were seen where the highest plankton concentrations were measured. The Deakos et el. (2011) study showed two individual manta rays travelling to nearby minor islands requiring a traverse of water up to 324 m depth and travelling a maximum distance of 40 km in 28h. The Yano et al. (2000) study in Yaenema used ultrasonic depth sensing transmitters with similar results to Clark (2008) in that mantas were found to swim around a depth of 10-30 m in inshore waters during the day and then swim offshore and remain at depths of 100-200 m at night but only a proportion of individuals returned to shallow waters the next day. Although this was not specifically a migration study, the results suggest the same

pattern of activity in Yaenema as at Komodo and Hawaii, with manta rays visiting shallow reef areas during the day and moving offshore at night. Initial studies of *M. alfredi* by Stevens, Clark and Rubin have indicated a similar pattern in the Maldives (G. Stevens, pers. comm.). Thus, all studies of *M. alfredi* populations have reported a similar diel pattern of movement indicating that *M. alfredi* spend the majority of their time in relatively deep water, especially during the night when they feed. The common names of inshore or reef manta ray are not suggestive of this pelagic lifestyle.

Photo-identification study of mantas (the population is known to include both *M. birostris* and *M. alfredi*) in Yaeyama, Miyako and Ogasawara islands (Yano et al. 1999a) showed that the mantas migrated between Yaeyama and Miyako islands, a distance of approximately 130 km. Migration from Yaeyama to Kerama islands (300 km) was also reported; based on the observations of T. Itoh, a professional diver, who identified the same manta rays at both sites (H. Ishihara, pers. comm.). Distances of up to 400 km between sites where individual *M. alfredi* have been sighted have recently been reported by Kashiwagi et al. (2010). These distances would indicate migratory activity by individual mantas in these locations**.** No explanation is given as cause for the migration (seasonal changes etc.) however some mantas also remained resident at Yaeyama during the same period providing evidence of simultaneously resident and migratory individuals. Similarly, in eastern Australia, Couturier et al. (2011) reported *M. alfredi* being sighted year round at Lady Elliott island but sightings at the more southerly sites (North Stradbroke island, Byron Bay) were mainly during the autumn and winter periods. 34 individuals were sighted in both areas and it was proposed that the movement was caused by seasonal changes.

In summary, research indicates that individual *M. alfredi* may travel tens of kilometres daily between preferred feeding and cleaning areas. If they return to the same areas on a regular basis throughout the year they might be considered **resident** (Homma et al. 1999; Dewar et al. 2008). If a manta is known to visit two sites, even if the sites are some 100 km apart, if they can be found at either site at any time then both sites would be considered within a 'resident' home range. However, if the probability of finding the manta at one of the sites becomes very low periodically due to a specific factor e.g. season change, plankton productivity, mating behaviours etc. then I propose they could be considered **migratory**.

Based on the definition of migration in the introduction (Kennedy 1985; Attenborough 1991; Dingle and Drake 2007), if the manta rays travel to different feeding or cleaning stations for part of a year whilst remaining absent from feeding and cleaning stations they visit during the other part of the year, they are considered **migratory**. The Maldives population appear to make a persistent migration from east to west sites with the change in monsoon seasons and the movements are predictable with the "to-and-fro" occurring seasonally, and the majority of the population is relocated. In the example of North Male atoll the distance travelled to the new locations is similar to that which they can cover in a single day (i.e. 40 km or less) but as they are absent from alternate feeding/cleaning stations during the period of their appearance elsewhere the Maldives population should be considered migratory.

Initial research of the oceanic migratory behaviour of populations of *Manta birostris* in the Revillagigedos Islands, Mexico, report migrations over distances of 613 km (one way) between groups of remote islands requiring a traverse of deep ocean (Rubin et al. 2008). Initial studies from La Plata Island, Ecuador of *M. birostris* indicate an oceanic migratory population as mantas are only sighted from June to September. It is unknown where they travel to or from outside this period, but no animals had been re-sighted from previous years from 2005 to 2009 (M. Harding, pers. comm.). Luiz et al. (2008) show *M. birostris* visiting Laje de Santos Marine Park in south-west Brazil in the austral winter. They proposed enhanced plankton productivity observed at the same time as the mantas were present as being responsible for attracting the mantas in a similar manner to zooplanktivorous basking sharks following their prey and actively selecting areas that contain high densities of large zooplankton above a threshold density (Sims and Quayle 1998). It is logical that mantas (of both species) are found where the highest densities of their food sources occur and will travel relatively large distances to forage. The infrequent survey rate is probably responsible for the low rate of re-sightings at these locations and it is difficult to make comparisons on re-sightings rates as they lack time element. However, individual *M. birostris* appear to be relatively infrequently re-sighted during a season or in consecutive seasons, unlike individual *M. alfredi* (Kashiwagi et al. 2010; Couturier et al. 2011; Deakos et al. 2011; Marshall et al. 2011; Kitchen-Wheeler et al. 2012). None of the seven *M. birostris* reported from the Maldives have yet been re-sighted. The migratory behaviour of *M. birostris* is less studied and appears to be different to that of *M. alfredi*. There appears to be consensus amongst researchers of manta rays that the movements performed by individual *M. birostris* i.e. long distance

movements (>>200 km one way) and non-annual return i.e. individuals would not be expected to return to the same site in a year is termed **Oceanic migratory** (Marshall et al. 2009; Couturier et al. 2011) (R. Rubin, pers. comm.). The term “pelagic migratory” should be avoided in describing movements and home ranges of mantas as all adult mobulids are considered pelagic (Couturier et al. 2012).

It appears the Maldives *M. alfredi* is the only recorded fully migratory population: migrating east to west across and between atolls in the Maldives; apparently following the movement of plankton caused by the changes in prevailing monsoon wind. Thus, they can be predicted to be sighted at cleaning stations on the side of the atolls which is leeward to the prevailing monsoon wind. There are some sites where mantas can be seen year-round but these sites are never directly windward and no individual mantas have been recorded as being seen year-round from these sites yet.

This study concurs with the hypothesis that presence of a nearby food resource appears to be the most important factor in predicting the presence of mantas (Dewar et al. 2008; Marshall et al. 2009; Anderson et al. 2011; Couturier et al. 2011; Marshall et al. 2011). Their presence would thus be less predictable during the monsoon changeover months when food distribution is more varied and in areas with variable plankton production or availability, often caused by geographical factors. The movement east to west across an atoll or between chains of atolls involves a travel of 20-150 km. Although manta rays will be typically observed on the lee-sides of the atolls, specific examples show that the migration route is not solely driven by availability of food with mantas moving between different cleaning stations along the lee-sides of atolls and even moving between atolls during a single monsoon period. This species is reported to spend much of its time travelling or feeding (particularly at night) over deeper water (Yano et al. 2000; Clark 2008; Dewar et al. 2008), so the common names of reef or inshore manta ray may be inappropriate. Some manta rays made much longer migrations north and south and between atolls, without evidence of a return to a specific area. These migrations are known to be of distances up to 270 km and across ‘open’ ocean. This suggests that movements of *M. alfredi* are not necessarily limited by bathymetric features. These movements could be considered Oceanic and confirm one-way migration distances of up to 300 km by *Manta alfredi*.

Chapter 6. Population estimates of Alfred mantas (*Manta alfredi*) in central Maldives atolls: North Male, Ari and Baa

6.1 Abstract

Manta alfredi is economically important in the Maldives due to the large number of tourists attracted to scuba dive and snorkel with them. It is important to understand the size of populations around main diving points and throughout the Maldives so that manta based tourism activities can be developed more successfully and the impact of tourism can be monitored. Using natural markings to identify individual mantas, Petersen and Jolly-Seber probabilistic estimators were used to assess population size for main dive sites and the central atolls of North Male, Ari and Baa. Between 2000-2009 1,835 individual mantas were identified from 3,373 sightings. There was a significant gender bias with a male: female ratio of 1:1.8. Only 24.7% of mantas were re-sighted between 1 and 34 times with a maximum gap of 7 years and 11 months between sightings. Estimates for populations around main dive sites in central atolls were 181-562. The population of North Male atoll was estimated at 537 (SD± 422). The results from atoll estimations were extrapolated to obtain an estimate of *Manta alfredi* population for the entire Maldives of 6,442 individuals.

6.2 Introduction

Estimates of abundance are essential for the assessment and successful management of fish populations (Zeller and Russ 2000). Mantas are not targeted in the Maldives, but they are commercially fished in many parts of the world, such as in Indonesia, India and in the Philippines (Compagno and Last 1999; Homma et al. 1999; Alava et al. 2002; Marshall et al. 2006), and very little is known about their migratory behaviour and population sizes worldwide. Two recent studies have estimated local populations of *M. alfredi.* Marshall et al. (2011) obtained annual populations of 149-454 individuals during a four-year study at a site in southern Mozambique and Deakos et al. (2011) estimated that up to 230 animals (from 290 individuals identified over 5 years) were using their study aggregation site in Maui, Hawaii, during 3-4 month sampling periods. There are currently no estimates of populations of *M. birostris* at any site.

The three atolls of this study (North Male, Ari and Baa) account for over 80% of tourist diver revenue, including 63% of regularly visited manta watching sites, and are currently the most developed for tourism. Annual tourist numbers have grown

consistently over the past decade (except for 2005, following the Indian Ocean tsunami on Boxing Day 2004) achieving 680,000 tourist entries in 2008. The increased intensity of tourism is expected to have some impact on the marine environment and there is awareness of the environmental issues and the need for sustainability within the tourism industry (Ministry of Tourism and Civil Aviation 2007). Although a very intense dive industry has been built up in the Maldives to observe these animals, to date no scientific study has been published on the size of the Maldivian *M. alfredi* population. The presence of mantas is often unpredictable and, since the size of their population is unknown, it is difficult to predict how the extensive tourism pressure will affect their normal behaviour. In order to keep the tourism business sustainable and to prevent extensive disturbance of manta populations it is important to estimate the size of the present manta population around the atolls and main tourist dive points, as well as to follow any changes.

The migration study reported in Chapter 5 was conducted at approximately the same time as this population study. Whilst it was known that a proportion of individual mantas were re-sighted at the same site, at other sites within the same atoll, or more rarely at other sites in other atolls, the results were unknown (i.e. whether there were atoll resident populations or not) so closed (Petersen 1896) and open (Seber 1982) population models were used to cover both possibilities. Petersen (1896) pioneered estimating population parameters using mark-recapture techniques and many studies have employed and developed these methods (Cormack 1968; Otis et al. 1978; Cormack 1979; Pollock 1982; Seber 1986; Schwarz and Arnason 1996). Petersen's method remains one of the most popular models for estimating the size of a closed population. The model requires that (1) there is no immigration or emigration; (2) all animals are of the same likelihood to be captured; (3) marks are not overlooked or lost/missed by observers and (4) the catchable population is the total population, while any portion of the population that is not subject to collection is not included in the estimate (Gatz and Loar 1988; Pollock 2000). It was suspected that the atoll population might be considered sufficiently closed as deletions from deaths or permanent emigration and additions from young adults were expected to be low or negligible over the relatively small timescales of these studies compared to the known lifespan of *M. alfredi* (35+ years). Marshall and Bennett (2010a) reported a significant proportion of mantas in southern Mozambique bearing scars from shark attacks. The great majority of individuals in the Maldives are unscarred (95% as reported in Chapter 4). Marshall and

Bennett (2010b) showed some mantas surviving even from several large bites. It is likely that mantas are not attacked as frequently by sharks in the Maldives as in Mozambique and most would be likely to survive, and in this study the losses from shark attacks were considered insignificant. Additionally, migrations outside of an atoll were reported as less than 1% of the identified mantas, as reported in Chapter 5. It is probable that there are cleaning stations currently unknown to us which are visited by a number of mantas so these animals would be missed in atoll population estimates (discussed further in Chapter 7).

Using photo identification instead of real capture greatly reduces the negative effect of trap response on the animals, therefore this limitation can be considered as not significant. The most serious limitation of this method is that different animals may have inherent variability in the probability of being sighted at survey sites. Factors which may affect capture probability include individual heterogeneity (preferences due to sex, size, age, shyness and boldness of individual animals) and temporary emigration (therefore, not a closed population). When animals exhibit heterogeneous capture probabilities, any available estimate of population size is likely to be markedly biased (Cormack 1968). The CAPTURE (open) model developed by Otis et al. (1978) which allows capture probability to vary with individual animal requires that large numbers of animals are caught in each sample and animals are caught a large number of times. When the experiments were devised it was known that (1) the majority of mantas were not re-sighted, and (2) there were frequently long periods between sightings; thus the commonly used models might not be useful.

The open model encompasses additions (recruits and immigrants) and deletions (deaths and emigrants) and is useful when studies are performed over longer period of time (years). The open model was developed independently by Jolly (1965) and Seber (1965) and required equal catchability and equal survival rates for all animals within each sampling time and this had to be assumed despite anticipated individual heterogeneity. The maximum time between samples used for the Jolly-Seber model study was 13 months (Lankan Reef/North Male atoll) and samples were taken in a single day, so relatively instantaneous. Since it is so difficult to know if assumptions are satisfied or not, both open and closed models were used to see which gave the most sensible results (see discussion).

By the end of the data collection we had a much better idea that individual mantas were atoll-based and had preferences for certain sites and that they migrated between west and east sides of the atolls with the seasons. Although a few (<1%) were reported in different atolls, the populations were investigated as being atoll-based. There were reports of mantas being sighted from all atolls in the Maldives (Anderson et al. 2011) so it was logical that each atoll has a population of mantas. The population study surveys were conducted in only three atolls and the results from the surveyed atolls were then extrapolated to estimate the total population size of *M. alfredi* for the entire Maldives.

6.3 Method

6.3.1 *Survey sites and methodology*

In order to minimise bias in population estimates caused by small samples, surveys were organised to obtain the largest likely sample size for each survey period, based on experience at the sites from observations in previous years of when manta numbers were likely to be at their highest. The study areas were well known fish cleaning station sites in North Male atoll (Lankan Reef and Boduhithi), Ari atoll (Table Thila and Madivaru) and Baa atoll (Nelivaru, Dhigu Thila, Donfanu Thila), regularly visited by mantas during the appropriate monsoon period as well as Hanifaru lagoon which is an important feeding area for manta rays and includes cleaning stations as well (Figure 6.1). The survey sites in this study varied in size and topography. North Male and Ari sites are described in Chapter 3. Nelivaru, Dhigu and Donfanu are submerged reefs or *thilas* and the cleaning stations are specific areas of the *thilas* and Hanifaru site is the south east section of the faro (Darwin 1842; Scheer 1972) or ring reef of the same name. At Hanifaru, the reef structure creates a secondary lagoon which traps out- flowing plankton and includes some coral bommies which are cleaning stations. For the purposes of this survey, the size of the site was considered irrelevant as the objective was to capture the maximum possible number of animals observed during each observation period (45-60 minutes). Two to six trained observers were deployed per survey to record relevant information of mantas to ensure identification of mantas encountered.

Two types of survey were undertaken: routine and intensive (n=329 and n=100 respectively). The routine surveys were conducted from December 2000 to August 2009; these were primarily during tourist diver visits to cleaning stations. Each involved a 45-60 minute scuba dive during which mantas were individually identified using the

methods described in Chapter 4. These surveys identified the majority of the animals visiting each site and were conducted when encounters with mantas were thought likely, based on experience. Approximately two surveys were made per week in the relevant season but the rate of survey varied from one year to the next.

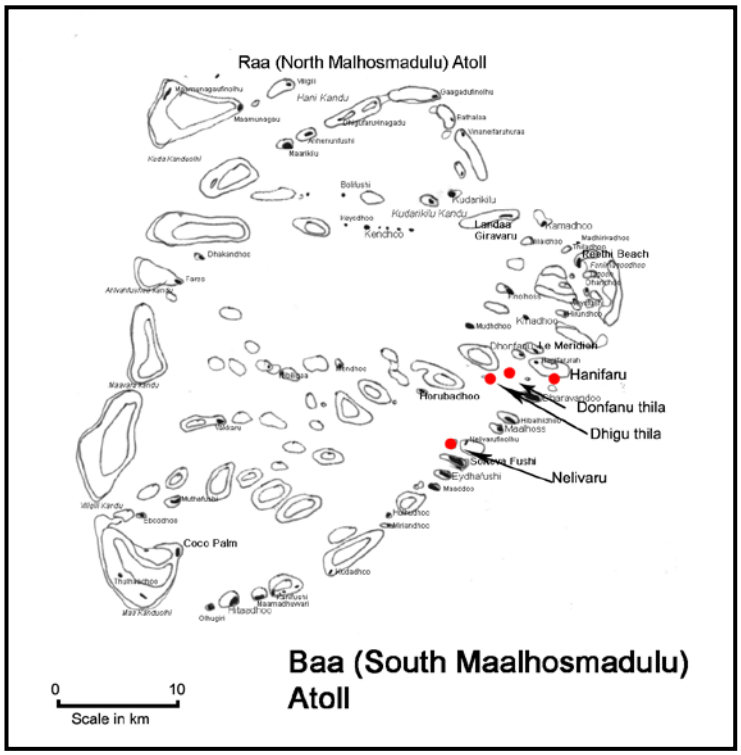


Figure 6.1 Diagram of Baa atoll to show locations of the cleaning stations Donfanu, Dhigu and Nelivaru thilas; and the feeding site of Hanifaru. All are located on the eastern side and are visited by mantas during the SW monsoon.

In addition, in 2006 at North Male atoll, in 2007 at Ari and North Male atolls and in 2009 at Baa atoll, one hundred intensive surveys were conducted. These were carried out during 23 full-day studies conducted at cleaning stations plus the Hanifaru feeding area. The studies were undertaken primarily to estimate population size. Survey dates for these studies were selected with the aim of obtaining as large a sample as possible on each day (the greater the number of animals identified the larger the number of re-sighted mantas likely) based on experience of the site and conditions. The time spent underwater by the diving team during the intensive full-day studies varied from 2 to 10.5 hours per day (comprising from two to fourteen 45-60 minute surveys), depending on conditions and manta activity. At Lankan Reef, Boduhithi and Madivaru the sites were surveyed continuously for 6-10.5 hours. At other sites surveys were only commenced if mantas were confirmed to be present during a reconnaissance by a snorkeler. Two to six observers were deployed per survey. Divers either waited nearby the cleaning station throughout the period (Lankan Reef, Table thila sites) or made transects of the reef to visit the different potential cleaning stations or feeding areas

(other sites). Surveys from the intensive full-day studies were combined to calculate population estimates using the method of Petersen (1896) and Jolly-Seber (Seber 1982; Seber 1986). Full day samples were used in all the population estimates to standardise the samples and so they might be considered comparative for effort.

The annual monsoon cycle affects manta movements, therefore mantas may be predicted to visit feeding and cleaning areas located on the east and west of atolls in the lee of the prevailing monsoon wind where productivity is high due to phytoplankton blooms (Anderson et al. 2011) as described in Chapter 3 and investigated in Chapter 5. Data were collected in both monsoon seasons in North Male atoll, being the only atoll with well-established manta cleaning stations on both sides (the other atolls have cleaning stations on both sides but mantas sightings are unreliable and thus the sites are rarely visited). By the time analysis was performed it had been established that many mantas which visited Lankan Reef also visited Boduhithi cleaning stations during the other monsoon and therefore came from the same population (see Chapter 5). Intensive surveys in North Male atoll were undertaken at Lankan Reef in July 2006 and July and August 2007 and at Boduhithi on three days during the NE Monsoon season of 2007, in between the Lankan surveys. All the surveys were considered as sampling the same population, and thus the North Male intensive surveys were carried out over a period of around 13 months. The intensive surveys in Ari atoll were conducted in February and March 2007, approximately a month apart. The surveys in Baa atoll were made on consecutive days from the 3rd to 18th August 2009, but no survey was possible on 14th and 15th for operational reasons. These surveys were made at Dhigu, Donfanu, Nelivaru and Hanifaru sites with the majority of time spent at the latter site. Typically, we surveyed cleaning stations in the early morning and then spent the period after midday at Hanifaru. The sites in Baa atoll were in close proximity (< 5 km apart except Nelivaru which was approx. 10 km distant) and on several occasions mantas would be first sighted at a cleaning station in the morning survey (including Nelivaru) and then be seen feeding at Hanifaru during the afternoon surveys. Observations from each day at the various east Baa atoll sites were combined as full-day studies, as atoll population was being investigated.

6.3.2 *Estimating population size*

Two approaches were used to estimate populations using Petersen's method (closed model). In the first, 'marked' individuals consisted of all the animals which had

previously been identified in that atoll (in both routine and intensive surveys) at any time earlier than the date when the population was estimated (method 1). In the second (method 2), pairs of full-days of intensive surveys were compared (e.g. A vs. B, A vs. C, B vs. C, etc.) with the marked population identified only from the earlier intensive survey day i.e. a two-sample method. The two days were up to ~13 months apart. Each pair provided an estimation of population and means of these results were then calculated and reported in Table 6.2. The period of sightings data used in method (1) varied depending on the days when the intensive full-day studies were carried out. For Lankan reef (North Male atoll) this involved mantas sighted between November 2001 and August 2007; for Boduhithi (North Male atoll) sightings between November 2001 and March 2007; for Ari atoll sightings between January 2002 and March 2007; and for Baa atoll between October 2007 and August 2009. The majority of sightings from North Male and Ari were obtained from 2003 onwards so most of the data used to provide the marked sample in the Petersen (1) method for these atolls was obtained over 3.5-4.5 years.

The Jolly-Seber analysis only used the 23 samples from the 23 full-day studies with all (2-12) surveys from each day pooled, thus sightings were a maximum of 13 months apart.

Discovery curves for the population of identified individuals for each atoll were also produced for each atoll. Number of surveys was used as the unit of measure. Surveys were carried out over varying periods of time (2-9 years at each atoll) and with long gaps between series of surveys when sites were windward or I was absent from the Maldives. Additionally the intensity of surveys per day varied with only one hour being performed on most days but up to 10.5 hours (12 surveys) performed on other days. Plotting against time or date would have produced very uneven curves.

The largest possible samples were required to reduce error as small samples often produced zero or low-numbers of re-sightings which cannot be used for population estimation. A day's worth of surveys were pooled in order to create a large sample (Bailey 1951). Other researchers of manta ray populations have pooled a number of surveys over periods of months and then performed analysis comparing "year" samples (Deakos et al. 2011; Marshall et al. 2011). This method was unnecessary because a day sample contained similar numbers of animals sighted in a season in the other areas (up

to 93 mantas were reported in a single day in this study) and made the sampling periods relatively instantaneous. Two different marked samples methods (method 1 and method 2) were used with the Petersen model as it was hoped that using the large 'all the animals which had previously been identified in that atoll' as the marked sample would provide the biggest possible sample of marked animals and avoid the errors in small samples, but this method would likely violate the assumptions of a closed population. This method was also likely most likely to be to be measuring the atoll population. Method (2) involved the comparison of discrete samples within a much closer time-frame and so was less likely to compromise the closed population assumptions. This method was likely to be measuring the population around the site. In all three methods, samplings were repeated where possible so that means could be calculated (Cliff et al. 1996; Castro and Rosa 2005).

The *M. alfredi* population for the entire Maldives was estimated using an extrapolation of the results of the surveyed atolls, with the populations being considered proportional to geographical area. The results in Chapter 5 indicated that individual mantas are atoll based, with less than 1% of individuals known to travel between atolls, and this study and Anderson et al. (2011) suggest that each atoll has a population of mantas. It is logical to assume that the size of the manta population is related to the size of the atoll. The 26 atolls were grouped geographically to create 'natural' areas e.g. Baa atoll would consist of the two main sections of Baa plus the much smaller Goidhoo atoll which is close by; Haa Alifu and Haa Dhaalu were combined as they are geographically the same atoll and include the much smaller Ihavandhippolhu and Maamakunudhoo atolls etc. This grouping of geographical areas created 17 atoll areas. The area was calculated for each. Petersen (1) is the only method which allowed relative abundance among 3 atolls to be determined and so was used to calibrate estimates based on best estimate for North Male using Petersen (2) and Jolly-Seber. These estimates were used to establish a population/area relationship using regression from the estimates of the three studied atolls. The population for each atoll was estimated from the regression relationship and the total population for the Maldives estimated from adding the estimated individual atoll populations together.

6.4 Results

All sightings data (3,373 sightings) of 1,835 individual mantas (range 1-35 sightings per animal) contained in the Access database were analysed for sightings of mantas in the

three atolls of this study. 97.3% of recorded mantas were observed in the three atolls investigated in this report. Only 17 out of 1,835 (entire database) individuals (≈ 1%) were re-sighted in different atolls (all movements between atolls involved the study atolls). As movement between atolls was low it was considered not significant in this study. The longest gap between sightings of a single manta in the three study atolls was 7 years 11 months, with 7 other mantas having a gap in sightings of over 5 years. A gap of >3 years was found to be relatively common (>100 examples).

The first population estimate (Table 6.1) was calculated using the known (marked) population for the entire atoll up to the date of the survey and calculated using the Petersen's method (1). In the table, the 'Total mantas' column is the number of individual mantas identified on that survey day and the 'Marked' column is the number which had previously ever been identified from the total. Based on this model the mean *M. alfredi* atoll population size for North Male atoll was estimated as 716 (SD ± 68.8) based on survey data from Lankan Reef, and 1441 (SD ± 465) based on survey data from Boduhithi. The mean site estimates for atoll population from Lankan Reef and Boduhithi were compared using one-way ANOVA-test to test variances in the result sets. The estimates are significantly different ($F = 10.07$, $P = 0.025$). Nevertheless they were combined as they are both estimates of a North Male population, producing a mean result of 1026 (SD ± 474) mantas. The mean estimated population for Ari atoll was 1468 (SD ± 634) mantas and mean estimated population of 719 (SD ± 631) mantas for Baa atoll, which is not significantly different to combined North Male result (one-way ANOVA: $F = 1.24$, $P = 0.281$). Summarised results of population estimates using method (1) are also shown in Table 6.2.

The estimates using Petersen's method (2) (two-sample method), when two full-day were compared with the 'marked' population including individuals identified only from the first sampling day, could only been used for North Male and Baa atolls because no mantas were seen on both of the full day sampling studies in Ari atoll. For Baa, only population estimates from full-day surveys when at least 10 animals were captured and at least one animal was matched on the paired dates were analysed. Small samples will cause a negative bias in estimates (Otis et al. 1978).

Survey Site	Survey dates	Total mantas seen that day	Marked mantas	Estimated population size (N)
Lankan (M)	18.07.2006	30	24	633
Lankan (M)	26.07.2006	66	50	685
Lankan (M)	31.07.2007	27	24	771
Lankan (M)	08.08.2007	72	64	774
				Mean 716 (SD ± 68.9)
Boduhithi (M)	09.01.2007	73	20	1956
Boduhithi (M)	09.02.2007	93	42	1315
Boduhithi (M)	24.02.2007	86	53	1051
				Mean 1411 (SD ± 465)
North Male atoll total				**Mean 1026 (SD± 474)**
Table Thila (A)	29.03.2007	55	22	1916
Madivaru (A)	02.02.2007	19	11	1019
Ari atoll total				**Mean 1468 (SD ± 634)**
Donfanu/Hanifaru (B)	03.08.2009	8	0	No calculation possible
Donfanu/Hanifaru (B)	04.08.2009	25	0	No calculation possible
Donfanu/Hanifaru (B)	05.08.2009	23	1	1564
Dhigu/Hanifaru (B)	06.08.2009	47	17	325
Hanifaru (B)	07.08.2009	18	3	744
Nelivaru/Hanifaru (B)	08.08.2009	11	4	382
Don/Dhigu/Hani (B)	09.09.2009	5	2	365
Hanifaru (B)	10.08.2009	10	5	298
Nelivaru/Hanifaru (B)	11.08.2009	8	3	411
Hanifaru (B)	12.08.2009	8	4	318
Hanifaru (B)	13.08.2009	46	14	536
Hanifaru (B)	16.08.2009	6	2	585
Hanifaru (B)	17.08.2009	12	1	2388
Hanifaru (B)	18.08.2009	34	10	714
Baa atoll total				**Mean 719 (SD± 631)**

Table 6.1 Estimates of *Manta alfredi* population size for North Male (M), Ari (A) and Baa (B) based on Petersen's method (1), using "Marked" population being individuals previously seen before in the atoll at any time prior to the survey date. (N: estimated population size; SD: standard deviation).

Based on this method the mean atoll population estimates were 330 (SD ± 243) for North Male atoll population based on Lankan Reef data; 563 (SD ± 159) for North Male atoll population based on Boduhithi data; 536 (SD ± 244) for North Male based on combined Lankan Reef and Boduhithi data (n=21 paired results, range 139-990) and 301 (SD ± 183) for Baa based on East Baa atoll sites data (n=23 paired results, range 68-850). The estimated mean population for North Male atoll using the combined sites

is 536 (95% CI 425-647). When the estimates of atoll population using Petersen's methods (1) and (2) were compared using one-way ANOVA the estimates for all sets of data were statistically different (Lankan Reef: F = 9.24, *P* = 0.016; Boduhithi: F = 9.56, *P* = 0.037; North Male combined: F = 12.97, *P* =0.001; Baa: F = 8.91, *P* = 0.005). Thus, population estimates using Petersen's method (2) were significantly smaller than estimates using method (1).

Using the Jolly-Seber method applied to the full-day studies of intensive surveys for North Male and Baa sites, the following population size (N) was estimated (summarised in Table 6.2): mean 181 (SD ± 70.7) individuals based on Lankan Reef data and 371 for Boduhithi data (single result). Using combined Lankan and Boduhithi samples (n=7) with the analysis performed on the samples in chronological order, the population estimate was 538 (SD ± 422). These samples were obtained over approximately one year so this result suggests that the upper estimate of population in North Male atoll in a year is 1172 using the Jolly-Seber method.

Model of population estimation	**Atoll**	**Mean Population estimate (N)**	**Range of estimates**
Petersen's method (1) (entire atoll as marked sample)	(Lankan) North Male	716 (SD ± 68.9)	633-774
	(Boduhithi) North Male	1441 (SD ± 465)	1051-1956
	(Combined) North Male	1026 (SD± 474)	633-1956
	Ari	1468 (SD ± 634)	1019-1916
	Baa	719 (SD ± 631)	298-2388
Petersen's method (2) (paired samples, earlier day as marked sample)	(Lankan) North Male	330 (SD ± 243)	139-810
	(Boduhithi) North Male	563 (SD ± 159)	381-990
	(Combined) North Male	536 (SD ± 244)	139-1222
	Baa	301 (SD ± 183)	68-850
Jolly-Seber	(Lankan) North Male	181 (SD ± 70.7)	131-231
	(Boduhithi) North Male	371 (only one result)	371 (only one result)
	(Combined) North Male	538 (SD ± 422)	139-1172
	Baa	204 (SD ± 163)	60-588

Table 6.2 Population size estimates (N) assessed for atolls and sites by different estimation methods.

Mean population estimates for North Male sites individually and combined using Petersen's model method (2) and Jolly-Seber were not significantly different (Lankan data: F = 0.67, *P* = 0.446; Boduhithi data: F =1.08, *P* = 0.407; combined: F = 0.00, *P* = 0.984). For Baa atoll sites using all fourteen intensive full-day survey results, the mean

estimate using the Jolly-Seber method was 204 (SD ± 163) and was not significantly different (F = 2.24, $P > 0.14$) to that obtained using the Petersen method (2).

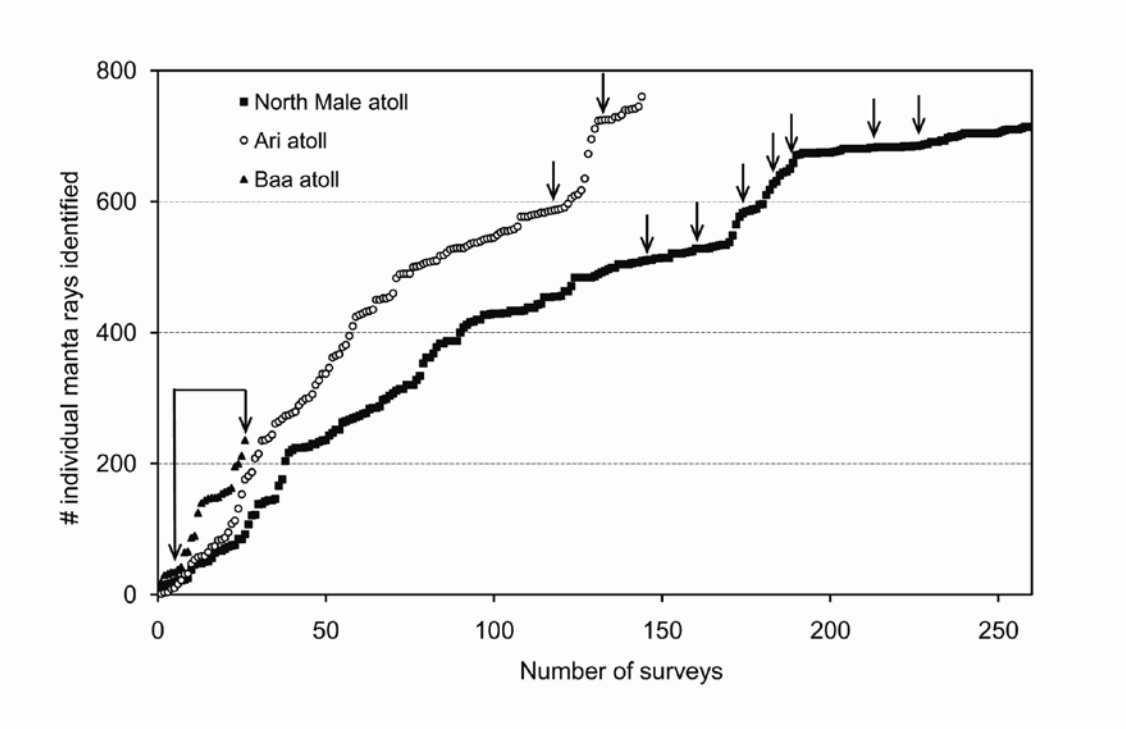


Figure 6.2 Discovery curves for new individuals identified in North Male, Ari and Baa atolls. New mantas are reported per survey in each atoll, in chronological order. Only the North Male curve shows an asymptote indicating that most mantas have been identified. Start of intensive surveys used for population estimates for North Male and Ari atolls annotated with arrows. Bracketed arrows show period of intensive surveys for population estimates in Baa atoll.

Discovery curves for the population of identified individuals for each atoll are shown in Figure 6.2. The number of new mantas discovered varied between surveys (range 0-38). For example, in North Male survey 143 (July) identified 17 new mantas, but the next day when 10 consecutive surveys were carried out, the most new mantas discovered in an hour was 4 causing the curve to flatten out again. The sequence of surveys 161 to 163 (January) in the North Male curve was for surveys at Boduhithi where 10, 17 and 12 new mantas were identified (respectively). Similar spurts in numbers of new mantas were observed around surveys 128 to 130 (March) at Table thila in Ari atoll (18, 38, 22 new mantas) and in the Baa curve when 21 and 33 new mantas were recorded during surveys 11 and 24 (August) respectively. The discovery curve for North Male appears to be reaching an asymptote suggesting that the majority of the atoll population has been identified in the 700+ already recorded. The regressions for Ari atoll and Baa atoll

were linear suggesting atoll populations far in excess of the 769 and 231 mantas currently known from these atolls, respectively.

To calculate an estimate of manta population for the entire Maldives, assumptions were made based on previous results. Assuming the restriction to a single atoll being probably caused by geography (the move to a different atoll would require a traverse of deep ocean and less than 1% of mantas were ever reported in different atolls) we may assume that *M. alfredi* are limited to the atoll areas as defined in the method. Mantas have been reported from all atolls (Chapter 5) therefore each atoll will have a population of mantas. The geographical grouping created seventeen atoll areas (Table 6.3).

The estimates of atoll population for North Male using Petersen's method (2) and Jolly-Seber were similar so was used as a baseline for an estimated atoll population i.e. ~537 animals to be used for extrapolation. The Baa atoll Petersen's (2) and Seber-Jolly results were suggestive of a population around a site, not an atoll and unfortunately we were unable to obtain a Petersen's (2) or Jolly-Seber estimate for Ari atoll. Thus only the Petersen's (1) method provided comparable population estimates for all three atolls that could be used to predict ratios of populations between Ari and North Male, and Baa and North Male that can be used to scale the Petersen (2)/Jolly-Seber estimate of North Male.

It was assumed that the ratio between Petersen (1) estimates for each atoll would be mirrored by Petersen (2)/Jolly-Seber, so that Petersen (1) values for each of the atolls can be used to estimate how much larger/smaller these populations will be relative to North Male. The Petersen's (1) estimate for Ari atoll of 1,468, based on proportion to the North Male Petersen's (1) estimate of 1,026, suggests an estimate for the population for Ari to be 537 x (1,468/1,026) = 768 mantas. This figure seems reasonable based on the discovery curve indicating the population for Ari atoll is over 769 known animals. Likewise the Petersen's (1) method estimate of population of Baa of 719 mantas can be used: 537 x (719/1,026) = 376 mantas. Using atoll populations for North Male of 537, Ari of 768, and Baa of 376 and regressing the three points (but forcing the intercept through zero, as zero area must have zero population) as shown in Figure 6.3 suggests the relationship Manta population= 0.2743 x Area.

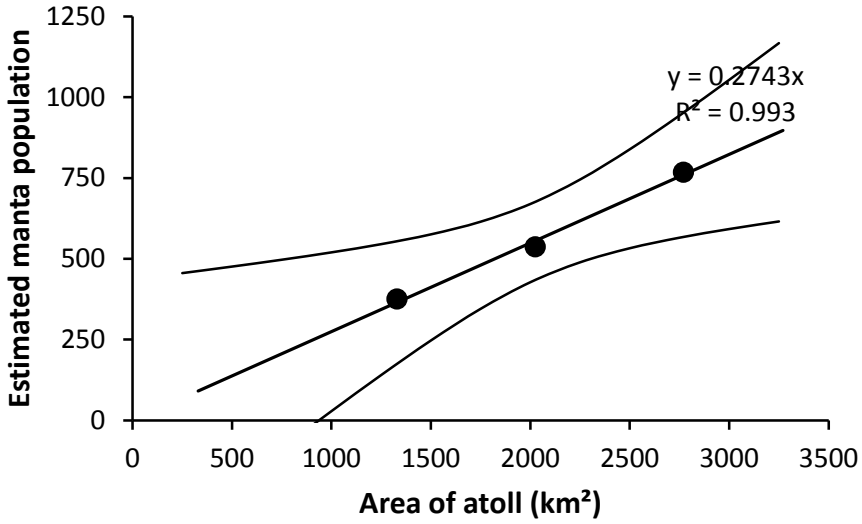


Figure 6.3 Regression of relationship between manta population and atoll area based on estimated populations for North Male, Ari and Baa atolls using more reliable Petersen's 2 and Jolly-Seber estimate for North Male atoll. The regression was forced through zero. The relationship Manta population = 0.2743 atoll Area was used to calculate estimated populations for each atoll, listed in Table 6.3.

This was used to estimate the atoll populations listed in Table 6.3. This result suggests the Maldives *M. alfredi* population is around 6,442 rays. The graph shows the 95% CL range which is very wide as there is only 1 degree of freedom to estimate 95% CL from standard errors for population estimates for each atoll area. If the upper 95% CL estimate for each atoll is used in calculations, the Maldives population is estimated at ~10,000. This might be considered an upper limit.

Area	Geographical combinations: atoll areas	Area (surrounded by reef)	Estimated population (= 0.2743 Area)
1	Haa Alifu, Haa Dhaalu, Maamakuudhoo atolls	1760 km²	483
2	Shaviyani	1859 km²	510
3	Noonu	1211 km²	332
4	Raa	1531 km²	420
5	Baa (north and south sections) Goidhoo atolls	1330 km²	365
6	Lhaviyani	863 km²	237
7	North Male, Kaashidhoo, Gaafaru atolls	2026 km²	556
8	South Male	514 km²	141
9	Ari, Rasdhoo, Thoddoo atolls	2770 km²	760
10	Felidhoo, Vattaru atolls	962 km²	264
11	Meemu	1092 km²	300
12	North Nilandhe	589 km²	162
13	South Nilandhe	723 km²	198
14	Thaa	1840 km²	505
15	Laamu	922 km²	253
16	Huvadhoo	3285 km²	901
17	Addoo, Foamullah atolls	208 km²	57
			Total 6,442

Table 6.3 Geographical combinations and area of each combination used to predict "atoll" population, which combined was used to predict an estimate of population for the entire Maldives of 6,442 mantas.

6.5 Discussion

Three different methods, with different assumptions were used to obtain population estimates. The Petersen (1) method produced the highest estimates; however the assumption of closure was likely to be violated for the North Male and Ari atoll estimates, in part due to the length of time over which the data for the 'marked' samples were gathered. The estimates are probably over-stating the populations, especially when compared to suggested populations from the discovery curves for these atolls. The estimates obtained from Petersen (2) and Jolly-Seber methods were similar for both North Male and Baa atolls. As the population estimates for North Male atoll were obtained using samples from both sides of the atoll and obtained over a 13 month period there is some confidence that they do reflect the annual population of the atoll. Castro and Rosa (2005) estimated the abundance of nurse sharks *Ginglymostoma cirratum* at Atol das Rocas off north-east Brazil using Petersen-Bailey and Jolly-Seber models and also obtained statistically similar results using both methods. This implies that the

Petersen (2) and Jolly-Seber method are estimating the same population whilst the Petersen (1) method is measuring a different population. In view of the greater estimates obtained using the latter, it may be an atoll superpopulation (i.e. the number of mantas visiting the atoll over the duration of the study) that was measured using Petersen (1).

6.5.1 *North Male atoll*

The difference in population estimates obtained from surveys at Lankan Reef and Boduhithi individually using all three methods suggest that different proportions of the atoll populations use the particular cleaning stations. This may be due to differences in the sizes of the cleaning stations. Lankan is a large bommie with approximately four areas of cleaners which can accommodate one manta each, so a maximum of four animals may clean simultaneously (although up to 20 other mantas may assemble around the cleaning station and wait for a cleaning station to become free). Boduhithi is a reef flat with dozens of cleaner fish areas and may accommodate 40+ animals being-cleaned simultaneously. Lankan Reef has a higher proportion of visiting females (63.5%) (data in Table 10.1, Chapter 10) compared to Boduhithi (54.9%): Yates corrected $\chi^2 = 4.087$, DF = 1, two-tailed $P = 0.043$. Significantly less mantas were seen at Lankan per survey than at Boduhithi (data in Chapter 7, table 7.7) based on Tukey-Kramer all pairwise comparison of number of mantas reported per survey at Lankan vs. Boduhithi. The mean size of female mantas was significantly larger than male manta rays generally (Chapter 4). Observations at Lankan Reef cleaning station reported a higher proportion of (larger) females. This may be because (smaller) males may face competition for access to the cleaning station.

Studies in other elasmobranchs have shown sharks display subordinate behaviour to individuals which are larger (Myrberg and Gruber 1974; Klimley 1983). I propose that competition may be the cause of the differences in population estimates and gender mix of mantas seen at Lankan compared with Boduhithi. At Boduhithi there are many areas of cleaner fishes, allowing more mantas to be cleaned simultaneously so smaller animals face less competition for cleaning station access and a higher proportion of the population (in particular smaller/male animals) are likely to be reported, resulting in the population estimates being greater for Boduhithi. Despite this, very few juveniles were reported at cleaning and feeding areas (at all sites, throughout all atolls) although they were occasionally seen, but could not be positively identified (they were easily disturbed), in shallow lagoons within the atolls. The population estimates missed these

juveniles and any individuals that do not visit the cleaning and feeding areas investigated, and so should all be considered as estimates excluding juveniles.
Due to the low level (<1%) of inter-atoll migration recorded, it appears unlikely that the higher population estimates using Boduhithi data alone is due to immigration of manta rays from outside the atoll during the NE Monsoon. Furthermore, almost 50% of mantas sighted more than once in North Male atoll were recorded at both Boduhithi and Lankan sites, alternating between them in the NE and SW Monsoons, respectively (as reported in Chapter 5) and this proportion was expected to rise with increased proportion of surveys at Boduhithi. It is likely that mantas from the same population visit both sites.

The population estimates determined using Petersen's method (2) and using the Jolly-Seber model based on Lankan Reef, and combined data were significantly smaller than using Petersen's method (1) (one way ANOVA: F = 7.71, P = 0.011; F = 6.77, P = 0.004 respectively). The mean estimates using the Petersen's method (2) and Jolly-Seber model on combined North Male data were 536 (SD ±244) and 538 (SD± 422). The Petersen's (2) and Jolly-Seber estimates were based on samples taken in a 13 month period so the atoll population could be considered closed. It appears that the Petersen (1) method is estimating a larger population and this is likely to be due to the assumptions of closure being violated by the length of time over which the marked sample was taken i.e. the superpopulation of the atoll. There are 709 mantas currently recorded from North Male and the discovery curve suggests that the North Male atoll population is unlikely to exceed 800 animals. The known migration level of <1% of mantas seen in more than one atoll over nine years is unlikely to cause population numbers to vary significantly between surveys conducted months, or even years apart. There is no obvious explanation for the difference between the Petersen method (1) atoll population estimates and the estimates obtained by the other methods.

Despite the large number of surveys, survey effort was low compared to the available time that manta s may visit cleaning stations and is estimated at around 2.5% of the time when mantas rays might be expected at the sites (the hours of daylight during the relevant monsoon season etc. See Chapter 7 section 7.3.5). However, up to 93 different mantas were positively identified visiting a cleaning station during an intensive survey day (it is likely that a few others were present but not identified) suggesting that almost 20% of the estimated North Male atoll population of ~540 may visit a small area of the atoll with cleaning stations in a day. With more intensive surveying it is likely that

individual mantas would be re-sighted more frequently. Taking these factors into account, the estimates of manta population in North Male obtained using the combined sites data using Petersen's (2) or Jolly-Seber models seems realistic.

6.5.2 *Ari atoll*

The discovery curve of new mantas for Ari atoll shows no clear sign of reaching an asymptote, with a recorded population of over 750 mantas. Estimates of the Ari atoll population could only be obtained using Petersen method (1) because only two full-day intensive studies were undertaken and no animals were seen on both occasions, thus precluding both Petersen method (2) and Jolly-Seber estimates. The lack of re-sightings was surprising because the surveys were undertaken only 30 days apart at two sites (Madivaru and Table Thila) approximately 42 km apart, which is within the known length of a daily swim by a *M. alfredi* (Dewar et al. 2008). This implies that movement between sites in Ari atoll is lower than in North Male atoll. According to the results in Table 5.2 only 122 (17.2%) of Ari based mantas were ever re-sighted and only 16 of these 122 animals were ever seen at different sites to that where first recorded unlike North Male based mantas of which 127 of 240 were re-sighted at more than one site. No data were available on SW monsoon cleaning stations so the results are confined to observations over the 5 months of the NE monsoon and sites located on the western side of the atoll. Seven mantas sighted in Ari atoll were also recorded in North Male during the SW monsoon. This result was consistent with the low level (<1%) of inter-atoll movement performed by mantas based in other atolls.

6.5.3 *Baa atoll*

There was a greater variation in the Baa atoll results than that observed in the other atolls which was unexpected since the intensive surveys were made on almost consecutive days so the population could be considered closed compared to those of the other atolls (196 of the 231 known mantas from the atoll were first discovered during the 16-day sampling period). Based on atoll area, the mean atoll population of 719 (SD ± 631) obtained using Petersen's method (1) for Baa atoll, is similar pro rata, with the estimate of 1026 (SD ± 474) for North Male atoll, which is about 1.7 times the area of Baa. The significantly lower estimate of 301 (SD ± 183) obtained using method (2) is due to small samples (~10) being relatively frequently obtained (in calculation, two samples of 10 may give a maximum population of 100). The Jolly-Seber population estimates were even lower than those obtained with Petersen's models (1) and (2) and

overall the three sets of estimates were significantly different (F = 7.46, *P*= 0.002). The small proportion of matches between surveys made only days apart and the wide range in population estimates suggest rapid circulation and dispersion of manta rays around this atoll. The mean estimated population using Jolly-Seber of 204 (SD ± 163), may be representative of the number of animals in the vicinity of Hanifaru (east Baa atoll) around the time of the survey dates, whilst the mean result of 719 obtained using Petersen method (1) may represent an estimate of the population of animals in the vicinity of the whole of Baa atoll. This result using Petersen (1) method might be considered more reliable than those made with this method for the other atolls as the Baa study was over a relatively short period of time (<2 years) so the population might be considered closed. The upper estimate of 588 by the Jolly-Seber method suggests that the majority of animals may have been in the vicinity of Hanifaru on that day. The discovery curve of newly identified individuals for Baa atoll is still increasing linearly with no evidence of any asymptote, indicating that the population is likely to be in excess of the 231 individuals so far identified.

6.5.4 *Implications for Maldives population estimates*

The 1,835 individually identified manta rays recorded from the central atolls of the Maldives is the largest group of individually identified *Manta alfredi* in the world so far. The extrapolated estimated Alfred manta ray population of the Maldives of 6,442 animals may be conservative but provides a starting point for managing this resource. The very tight linear relationship between atoll area and population gives confidence in the method. The upper limit (from 95% CI) of ~10,000 animals should be used cautiously as there is a low chance that populations in all atolls would be of the maximum population estimate.

In Mozambique, Marshall et al. (2011) re-sighted 40.5% of 449 individually identified *M. alfredi* and estimated annual population sizes of 149 to 454 individuals and a superpopulation (defined in the report as the number of mantas visiting the study area over the duration of the study) of 802. The study was conducted over four years and gaps of up to almost 3.5 years occurred between sightings. The relatively infrequent re-sightings and long gaps between sightings are a common feature of the two studies. In contrast, Deakos et al. (2011) re-sighted 73% of their identified population of 290 manta rays in Maui, Hawaii. They estimated the annual population using the program CAPTURE with Cormack-Seber-Jolly model as 77-230 animals. In these studies, the

maximum annual population estimate was close to the total number of identified animals (454 vs. 445 and 230 vs. 290 respectively).

The re-sightings rate in this study of 24.7% was the lowest of the three study locations and was probably due to the large geographical area covered in this study (the three atolls have a total area of approximately 6,126 km^2), and that regular study in Baa atoll had only commenced in 2007. The highest rate of re-sighting (34.5%) was in North Male atoll where greatest survey effort was expended. Individual heterogeneity may include behavioural differences, segregation by sex, age, size etc. and differing amounts of time spent in the survey area (Buckland 1990; Guttridge et al. 2009a). There was likely to be variation in capture probability and transience due to temporary emigration to other feeding and cleaning station sites within the atoll so the animal was unavailable for sampling. Individual heterogeneity in capture probability may result in increased re-capture of some individuals which negatively biases population estimates and vice-versa. The primary underlying assumption of the Jolly-Seber model is that there is equal catchability of marked and unmarked individuals (Schwarz and Arnason 1996). It is likely that both the differences in behaviours by individuals (the majority of mantas were only sighted once, but some mantas were sighted relatively frequently) and transience (even surveys on consecutive days produced low match rates) mean that this assumption was violated. It seems that marked animals are more likely to be re-sighted than an unmarked animal. These issues will cause a negative bias on population estimates. The two cleaning stations at Lankan Reef and Madivaru are very frequently visited by tourist scuba divers and it is likely that the mantas which visit the cleaning stations have become habituated to the presence of divers (see Chapter 7) but conversely, that other rays do not attend the cleaning station as they are deterred by the presence of divers. The effect may be cumulative, with fewer manta rays from a smaller population of bolder animals visiting year on year. This may be a cause of the difference in estimated atoll population between Lankan and Boduhithi (North Male atoll), and between Madivaru to Table thila (Ari atoll). Madivaru is the most valuable manta cleaning station in the Maldives in terms of revenue in dollars (Anderson et al. 2010). It is notable that Madivaru had the highest proportion of female mantas (~70%) of all the survey sites which were cleaning stations (Hanifaru is principally a feeding site). This may be due to the number of visiting divers deterring all but the boldest mantas from visiting the site. Whilst this is unlikely to have a detrimental effect on the animals as there are likely to be many other cleaning stations nearby which are less accessible to

divers, there may be an impact on income from tourism if well-known sites cannot be relied on for good sightings of mantas. It is in the interests of both the mantas and dive operators that tourism at the important sites is managed.

The estimates of manta population for atolls and cleaning/feeding sites may be used, broadly, as a baseline for future studies on the impact of tourism at the main Maldivian dive sites. Local (i.e. site) populations should be measured and monitored as well as atoll populations. Numbers should be measured over relatively long periods of time i.e. over a number of years, to take into account the natural fluctuations reported in other studies (Deakos et al. 2011; Marshall et al. 2011). It would be useful to conduct both site related studies, investigating populations observed at the sites (Lankan Reef, Boduhithi, Hanifaru etc.) annually, but to look for trends in numbers of mantas observed. Medium term trends (10+ years) should be investigated as results from this study have indicated a wide variation in the number of mantas observed at any particular site from one year to the next. With more year-sets of data, program CAPTURE could be used to analyse pooled year sets. The Ari atoll population requires more investigation. In the short term, experiments should be prioritised to full-day observations so that a useful Jolly-Seber analysis for both sites (Madivaru and Table Thila) and the entire atoll can be made. The apparent low level of movement by mantas between these sites could also be investigated further from that study. Before tourism expands extensively to the outer atolls it would be valuable to obtain population estimates in the accessible atolls of Addu, Huvadhoo, Haa Alifu and Haa Dhaalu where manta sites are already known.

The study of *M. alfredi* in Komodo Marine Park, Indonesia by Dewar et al (2008) indicated high site fidelity (the same mantas visiting the park on an almost daily basis, throughout the year), but this is not what we experienced during consecutive day long observations included in this study. This was particularly apparent on the study of the east Baa atoll sites and of Hanifaru in particular where the majority of Baa atoll mantas were reported. The sites were visited on consecutive days yet there were a low number of individuals re-sighted during the study, even on consecutive days despite large numbers of mantas being reported. This pattern is seen in the other atolls in the relatively high proportion of manta rays sighted once only. Instead, the low frequency of visits to the cleaning stations or feeding areas observed in this study suggests that there are likely to be many other cleaning or feeding areas of which we are currently

unaware (as the animals are likely to feed and be cleaned daily, see Chapter 7 for the investigation of this hypothesis), and that individual *M. alfredi* are highly mobile in the Maldives making accurate population estimation difficult. Wide dispersion has conservation considerations in slowly reproducing (estimated annual fecundity <1 from investigation reported in Chapter 10 and Marshall and Bennett (2010b)), fishery-targeted species. Fortunately there is no targeted manta ray fishery in the Maldives and the rays do not appear to travel much beyond an atoll but conservation and management of manta ray populations in other areas will be essential to prevent local over-exploitation of this species.

Chapter 7. The cleaning ecology of Alfred mantas (*Manta alfredi*), in the Maldives

7.1 Abstract

Despite an important manta-diving industry being based on the predictability of mantas visiting cleaning stations in the Maldives, and recreational diving literature describing the behaviour of mantas at cleaning stations; there was no previous scientific study of either *M. alfredi* or *M. birostris* as the client (being-cleaned) at the commencement of this study. *Thalassoma amblycephalum* appears to be the most important cleaner of mantas in the Maldives (based on its frequency and abundance), and occurs in large and numerous aggregations at certain sites e.g. Boduhithi and Table Thila. *T. lunare* was present in both its adult and juvenile forms as a primary cleaner at over 50% of sites and this study appears to be the first documentation of juvenile *T. lunare* being an important cleaner. There were five main species of fish identified as cleaners of mantas at the cleaning stations surveyed: *T. amblycephalum, T. lunare, Labroides dimidiatus, L. bicolor*, and *Bodianus diana*. No relationship between size of cleaning station or number of cleaners present and number of mantas using the cleaning station was established.

Individual mantas visit cleaning stations frequently, most likely daily. The time spent at a cleaning station in a day varied from 5 minutes (minimum recordable) to over 3 hours (mean 35 minutes, median and mode 20 minutes). Generally, state of tide, time of day and number of other mantas present could not be used to predict the number of mantas encountered but may be useful on a site-by-site basis. However, anecdotal evidence indicated that strength of current (which can be related to state of tide) could be a useful predictor of number of mantas present, but again, the effect may be site specific. A specific pose with the cephalic fins held loosely open was adopted by all mantas prior to, and whilst being-cleaned. This pose was termed the “open-cleaning” position in this study. Mantas swam significantly more slowly when being-cleaned than when feeding and a swim speed of around 16 wing-beats per minute should be considered diagnostic of cleaning behaviour. Some mantas appeared attracted to exhaled divers’ bubble streams, hovering in them to maximise exposure. This behaviour is performed by the majority of mantas observed. It is likely that the bubbles dislodge skin and parasites and could be considered a learned cleaning behaviour.

7.2 Introduction

In the Maldives, recreational scuba divers commonly observe Alfred mantas *Manta alfredi* at cleaning stations and an important "manta-diving" industry (snorkelling and scuba) is based on the predictability of mantas visiting cleaning stations. Recreational diving literature has described some manta behaviours at cleaning stations (interactions with cleaner fish, hovering and slow swimming movements) (Harwood and Bryning 1998; Rogerson 2004; Godfrey 2006), but there was no previous scientific study of either *M. alfredi* or *M. birostris*, or any other mobulids, as the client (being-cleaned) at the commencement of this study. There are short reports of client behaviour by other large marine animals (marine iguana, shark and turtle) by Hobson (1969), Sazima and Moura (2000) and Heithaus et al. (2002). They report the animals resting on the bottom nearby areas of cleaner fish and being cleaned by a variety of small wrasse and gobies.

Cleaning behaviour where one or a number of animals groom a client is most common and widespread in reef fishes (Losey 1972), and has been widely researched in fish since Feder (1966). Cleaners are defined as reef fish or crustaceans involved in the mutualistic relationship of parasite removal and wound cleaning of the client fish. The cleaner may remove ectoparasites, bacteria, mucus, diseased and injured tissue and unwanted food particles from the client (Feder 1966). The interactions usually occur in a defined area or territory, commonly referred to as a 'station' (Gooding 1964; Feder 1966; Youngbluth 1968). Thus, a cleaning station is a defined area of cleaner fish, although groups of conspecific cleaners may form large aggregations which are not fixed and move around a section of reef creating a movable cleaning station. Client fish visit the station in anticipation of being-cleaned by the cleaner fish. The association between cleaners and clients is widely viewed as obligate, co-evolved and mutualistic (Hay et al. 2004). At least 131 different species of fish (Cote 2000) undertake the role of cleaner with different species being the main cleaner of a specific client species in different regions. The diving literature listed in the first paragraph includes mention of the bluntheaded wrasse *Thalassoma amblycephalum* (Bleeker, 1856), the bluestreak cleaner wrasse *Labroides dimidiatus* (Valenciennes, 1839) and the moon wrasse *Thalassoma lunare* (Linnaeus, 1758) as cleaners of mantas. There is a short monograph in Debelius (1993) of bluntheaded wrasses cleaning manta rays but the species does not appear to be reported as a cleaner in other scientific literature. This study identifies the species of cleaner fish and their relative importance at a series of manta cleaning stations in the Maldives.

A review of fish cleaning literature relevant to this study identified the following conclusions:

1. Frequency of visit to cleaning stations by clients does not appear to be dictated by a high parasite or mucus load (Arnal et al. 2001) and may be more to do with tactile stimulation than actual cleaning (Bshary and Wurth 2001).
2. Clients chose the most effective cleaners if they had a choice of cleaning station (Bshary and Schaeffer 2002).
3. There is no relationship building between cleaners and clients which have a choice in cleaning station; instead, cleaners will prioritise their attention on the perceived highest predator (apparently, the biggest fish), attending the cleaning station (Bshary 2002).

Whilst these studies were of *L. dimidiatus* and *Elacatinus*. spp (which are very similar in appearance to *L. dimidiatus*), the conclusions were drawn from interactions with a number of client species.

There are surprisingly few records of reef fish cleaning elasmobranchs in view of the frequency of observation by recreational scuba divers of sharks and their activities. Feder (1966), Lewis (1967) and Randall (1958) reported that members of the Echeneidae (remoras) preyed on external parasites present on free swimming sharks, but the first report of sharks altering their behaviour to be cleaned was by Keyes (1982). He noted that sicklefin lemon sharks and lemon sharks (*Negaprion acutidens* (Rüppell, 1837) and *N. brevirostris* (Poey, 1868)) in an aquarium environment opened their mouths whilst resting on the floor to allow entry by a cleaner wrasse (*L. dimidiatus*). He also described a bull shark *Carcharhinus leucas* (Müller & Henle, 1839) assuming a head up swimming position at a 45º angle to the horizontal and slowing its swimming speed considerably whilst the wrasse investigated its head area. Sazima and Moura (2000) described Caribbean reef sharks *Carcharinhus perezii* (Poey, 1876) resting on the bottom to allow cleaning by the yellow nose goby *Elacatinus randalli* (Böhlke & Robins, 1968) and interpreted this as an adaptive behaviour specific for cleaning. Southern stingrays *Dasyatis americana* Hildebrand & Schroeder, 1928 were described being-cleaned whilst swimming slowly or resting on the bottom and adopting a stereotyped pose (Snelson et al. 1990). Gasparini and Floeter (2001) recorded *Thalassoma norohanum* Boulenger, 1890 and an undescribed species of *Elacatinus* as cleaners of *Carcharinhus perezii* and *Ginglymostoma cirratum* (Bonaterre, 1788) at Fernando de Noronha, off Brazil, but there is no discussion of behaviour. The visits by large schools of scalloped hammerheads *Sphyrna lewini* (Griffith & Smith, 1834) to the

seamounts of Cocos and Galapagos to be cleaned are widely reported e.g. Seifert (2001) in an article about hammerhead massings. The hammerhead sharks appeared to pose by rolling at a 45º angle and then ceased swimming for a few moments as they approached cleaning stations. This appears to be an example of another elasmobranch species which gathers in relatively large numbers (suggestive of social activity as part of the cleaning process) and swims to a specific cleaning station rather than passively resting on the bottom and waiting for cleaners to visit. These reports indicate a range of different cleaners of elasmobranchs (particularly small wrasses and gobies) and establish posing as a common precursor to cleaning.

A recent investigation of tide-related periodicity of manta rays and sharks at cleaning stations in the Coral Sea and outer Great Barrier Reef, Eastern Australia by O'Shea et al. (2010) reported sharks maintaining a vertical posture whilst being cleaned and *Manta alfredi* (reported as *M. birostris*, but identified as *M. alfredi* from photographs) engaging in being-cleaned for 5 minutes to 5 hours per day with the majority of cleaning activity for both sharks (49%) and mantas (59%) occurring on ebb tides.

The objective of this study was to document the many facets of manta cleaning ecology including identification of the main cleaner fish species observed cleaning mantas at the principal manta cleaning stations. The study investigated (a) how many cleaners clean a manta, (b) how many cleaners there were at a cleaning station, (c) how long a cleaning session lasted for each manta and (d) how frequently individual mantas visited a cleaning station. External influences on manta cleaning activity were also investigated including time of day and the effects of tide and current. These influences were investigated in an attempt to explain the variations in manta numbers observed and provide direction for future experiments. Typical behaviours by mantas whilst being-cleaned, including a measure of swim speed (to compare with speeds during other activities), posing and movements around the cleaning station, were described or established as they have not been reported previously. Quantitative measures of behaviour e.g. mean time spent cleaning during a cleaning period and also in a day (from multiple sessions) and how many mantas visited a particular cleaning station during daylight hours were also investigated in order to provide some insights on manta behaviour and ecology.

7.3 Methods

In preparation for surveys, all participants were trained on individual manta identification through lectures and practice dives observing mantas. The level of experience of the participants varied from experienced field observers with hundreds of hours of observation dives logged, to inexperienced first time observers. The teams consisted of 2-6 observers led by an experienced guide with detailed knowledge of the site. The teams were organised to balance experience in order to maintain quality of data collected. The guide/leader used underwater photographic equipment to record the activity. Other members of the team (volunteer researchers) used recording materials (including cameras, videos and prepared slates) which they had practised with.

Eight days of consecutive surveys were conducted at Lankan (4 days), Boduhithi (3 days) and Madivaru (1 day). The consecutive surveys consisted of a series of 45 or 60 minute periods during which a team was deployed to record manta activity (identify individual mantas and record their arrival and departure where possible) and count or identify cleaner fish interacting with mantas (see below for further details on what was recorded). As each period was consecutive to the previous, this achieved a continual survey of the cleaning station for periods of 6 to 10.5 hours per day. The cleaning stations are known not be used by mantas at night (pers. obs.) so observers were only deployed from 06:45 to 17:30. Observation days were selected, from experience of the site, for optimal current and tide conditions likely to result in good manta observations whilst including a variety of conditions. As observations were carried out over periods of up to 10.5 hours the majority of a tide cycle was covered in most of these surveys and a range of current conditions were experienced. The information recorded included:

1. Identification of individual mantas present (see Chapter 4 for method).
2. Arrival and departure time of each individual manta.
3. Species identification and count of cleaner fish observed cleaning mantas (methods to count cleaner fish are described in cleaner fish surveys section).
4. Estimate of current strength checked by surface hand-held GPS, measured in nautical miles h^{-1} and converted to $m.s^{-1}$ or by distance travelled with time method. Note that the current in the areas occupied by mantas was often different (usually less, as the locations were sheltered) to that experienced on the main reef and could not easily be measured without disturbing the mantas.
5. Video recording of manta behaviour at or nearby the cleaning station.

Divers entered the water up-current from the cleaning station five minutes before the observation period was due to commence in order to allow time to find the cleaning station and settle into position. The team was requested to collect as much data as possible, with priority given to obtaining accurate identifications of individual mantas. Thus, if five mantas were seen, the observers were asked to ensure that each animal was logged fully (one at a time, see Chapter 4) before collecting other data. When large numbers of mantas were present it was very difficult to obtain the necessary information required to identify every single animal and on occasion, some may have been missed. Teams were requested to remain at the observation site whether or not mantas were present. At the end of each 45/60 minute observation period each team was replaced by a new team. Video and photo data were stored on computer and notes from slates were transferred to report sheets and ultimately onto the Access database.

Cleaner fish population surveys

Surveys of cleaner fish populations (assessed to be cleaning mantas) were only carried out at Lankan reef site (section 3.5.1) on one day (18.7.2006) over ten 45-60 minute surveys. Slates were pre-printed with a list of cleaner fish species likely to be encountered and observers were asked to record the number of fish for each species, seen cleaning a manta (fish were assumed to be cleaning if they were on or around a manta at time of count) in a one-off count during each survey. As fish were continually moving during surveys it was difficult to make an accurate count of numbers of individuals of each species present. Additional counts were made from analysis of photos and video footage (Figure 7.1) and this latter method was used to analyse data from other cleaning stations. This latter method would be likely to be underestimating the number of cleaners of each species; hence the results represent minimum numbers.

Manta behaviour surveys

The length of time that mantas spent being-cleaned was measured by recording the arrival and departure time of each identified manta to the cleaning station area, and this activity was a priority for the consecutive survey days. At Lankan it was possible to monitor most mantas present at the cleaning station at any one time as it consisted of a single block which could be continuously monitored. Mantas present were monitored in 5 minute intervals and actual times of arrival and departure recorded. It was more difficult to report times of arrival and departure of mantas at other the sites so the time when a manta was identified was reported and comments about departure or return were

made. The time when the mantas were present at Lankan reef during the four continuous survey days are reported in Appendix C with analyses in the results section.

It became apparent that the amount of time when cleaner fish were actually cleaning a manta was only a proportion of the time when the mantas was in the vicinity of the cleaning station (see section 7.6.2). For this study, a manta was deemed to be involved in cleaning activity if the manta had open cephalic fins and remained in the vicinity of the cleaning station (including short swims away from the cleaning station, as long as it returned within 5 minutes). Counts of mantas at the cleaning station at any one time, or per day, were derived from arrival and departure observations (Appendix C).

Cleaning frequency study

Using data on the median/mean/maximum number of times a manta is sighted at a particular survey site it is possible to estimate the frequency of visit. This method is the same as was used in Chapter 5 in order to determine how often an "average" manta would visit a cleaning station. Assumptions for each site had to be made including length of season and number of hours each day mantas might be cleaning. The assumptions for each site are listed in the results section. Using data on number of surveys conducted, success rate in observing mantas (Table 5.1 Chapter 5), the frequency of visit for a "typical" (median/ modal sighted and mean sighted) manta and most frequently sighted manta was derived for each site. The sample of mantas for each site on which the predictions were made was the first 50 mantas recorded for each site as they had the longest history, unless the total recorded number of mantas was less than 50 in which case the entire dataset was used.

Manta behaviours whilst being-cleaned

To investigate posing behaviour, swim speed, and movement around cleaning stations, videos were analysed to identify common and unusual behaviour (activity rarely seen but apparently important). All surveys (n= 381) were analysed for data on individual manta sightings however only the surveys conducted (or supervised) by the author (n= 325) were analysed for number of mantas seen and identified during each survey.

It is likely that the presence of scuba divers will have some impact on the natural behaviour of mantas but every effort was made to minimise diver-manta interaction.

However it appears that one of the diver-manta interactions has become part of the mantas' cleaning process and this is also reported.

Figure 7.1 Analysed photograph of manta at Table thila with cleaners counted. Each red dot is a *T. amblycephalum* and each yellow a *T. lunare*. This manta has 67 *T. amblycephalum* and one *T. lunare*. The *Odonus niger* (Rüppell, 1836) in the photograph are not known as cleaners of mantas and are feeding on phytoplankton in water column.

Analysis of behaviours

Data from the Lankan consecutive surveys on time spent cleaning per session and per day were analysed using Minitab in order to identify mean and median measures. Additionally, the mean number of mantas observed per survey at the different cleaning stations was compared to identify the most frequented cleaning stations.

In order to predict the optimal observation period for mantas at cleaning stations it was necessary to investigate the possible influences on mantas being present, e.g. time of day, tide effects, current effects. Manta activity during consecutive surveys at Lankan and Boduhithi were investigated in more detail as these are the most accessible sites but it is likely that conclusions drawn from these sites may be applied to other cleaning stations. Influence of tidal status was investigated by using predicted tide based on the tide position at Male (from tide Tables or using AutoTide tide prediction software) to manta sightings data for the relevant date and time. The number of mantas reported each 5 minutes was reported at Lankan so the results of number of mantas sighted were investigated for the influence of time of day and state of tide using a Rayleigh test. For Boduhithi and combined data (all sites/survey) the effect of state of tide was

investigated by dividing tide-state into six 2-h categories: high, high-falling, falling-low, low, low-rising and rising-high. The state of tide was determined by comparing the time of survey with the tide position predicted. The mean numbers of mantas reported per tide state were compared to determine if there was a significant variance in numbers of mantas seen at each tide state. This analysis was performed to see if there was a relationship between the number of mantas with tide at a single cleaning station (Boduhithi) and a general relationship (combined data).

The number of mantas present at any one time may influence the length of time that each individual spends at the cleaning station (or site). The presence of more mantas may increase the time spent as they take longer to be cleaned, or alternately the presence of a greater number of mantas may cause them to spend less time due to competition for access etc. The relationship between time spent by an individual manta in a day at Lankan Reef site with (1) the total number of mantas also being cleaned during the same cleaning period and (2) the maximum numbers of mantas at the site at the same time as the individual manta were investigated. The sample was the 132 cleaning periods recorded on 18.7.2006 and 26.7.2006 at Lankan. For example, on 26.7.2006 manta 1298 L398 made three separate visits (three cleaning periods), totalling 75 minutes (see appendix C). During the first period 11 other mantas came and went (total number identified during same period), but a maximum of 8 mantas were present at the same time as 1298 L398. During the second period a total of 8 mantas came and went but a maximum of 7 were present at the same time, etc. A regression of time spent at cleaning station by an individual manta with number of other mantas for both measures (methods 1 and 2) was determined using Minitab.

Swim speed (beats per minute bpm) during ‘being cleaned’ and ‘feeding’ behaviours were compared to determine whether there was a significant difference which might be used to objectively differentiate behaviours (‘being-cleaned’ and ‘feeding’ can also be differentiated from cephalic fin positions investigated in section 7.6.8 and the absence of cleaning fish when feeding). The method of swim speed measurement used was described by Yano et al. (1999b), with one complete cycle of pectoral fin movement from lowermost deflection to uppermost and back to lower, defined as one beat. In this study, a measure of beats per minute is used for comparisons.

Results for the cleaner fish studies and the mantas being-cleaned studies are reported separately. The cleaner fish studies are reported as section 7.4, followed by a conclusions section from the cleaner fish results only (section 7.5). Results from the mantas being-cleaned studies are reported in section 7.6. The results from both studies are discussed in section 7.7

7.4 Results and observations from cleaner fish studies

7.4.1 *Cleaners of mantas*

Cleaning station	*Thalassoma amblycephalum*	*Labroides dimidiatus*	*Labroides bicolor*	*Bodianus diana*	*Thalassoma lunare*	*Echeneis naucrates*
Lankan	P	S		S	S	S*
Boduhithi	P	S				S*
Sunlight	P	P	S		P	S*
Fairytale	S	P	S	S	P	S*
Table Thila	P	P				S*
Kalhahandi	P	P	S			S*
Madivaru	P	P		S	P	S*

Table 7.1 Primary (P) and secondary (S) cleaner fish observed cleaning mantas at main survey sites. Classification was primary if abundant and/or first to clean or secondary if infrequently sighted cleaning or not always present. * *E. naucrates* is carried by the mantas and not resident at the cleaning station.

All survey sites contained of a number of aggregations of cleaner fish (fixed or moving, single or mixed species). As a single manta may pass over any one, or all, of the aggregations at a site during a single cleaning session, the aggregations may be considered part of the same cleaning station. At Lankan and Sunlight the aggregations were so close that it is likely that individual cleaner fish re-locate from one aggregation to another during a day, and at Boduhithi and Madivaru the aggregations were observed drifting around the reef, acting as a moving cleaning station. The cleaning station was identified by the aggregation of cleaner fish. No crustaceans were observed cleaning mantas at any location in the Maldives at recreational diving depths.

Area	Species	Mean count (*n*=10)	Comments
1	*T. amblycephalum* *L. dimidiatus* *B. diana* *T. lunare*	60.0 (SD ± 17.32) 3.8 (SD ± 0.42) 3.9 (SD ± 0.32) 5.5 (SD ± 0.97)	This was the congregation of cleaners most frequently visited by mantas, estimate 90% of mantas attempted to clean at this area. See Figure 7.15 for swim pattern of a manta.
2	*T. amblycephalum* Adults *L. dimidiatus* *T. lunare* adults	29.8 (SD ± 5.47) 0.2 (SD ± 0.42) 3.0 0.2 (SD ± 0.42)	This was the second most commonly visited area
3	*T. amblycephalum* *L. dimidiatus*		None seen on date of surveys but reported on other dates
4	*T. amblycephalum*		None seen on date of surveys due to the presence of divers but seen on other dates

Table 7.2 Number (mean and standard deviation SD) of each species of cleaner fish reported at Lankan Reef main cleaning station bommie during 10 surveys conducted on 18.7.2006.

Site	**Species**	**Mean number of cleaners observed cleaning mantas (from analysis of photographs and video)**	**Comments**
Sunlight Thila	*L. dimidiatus* *T. lunare* *T. amblycephalum*	0.63 (SD±0.89) 0.94 (SD±0.75) 8.19 (SD±7.67) *n*=16 photo and video sequences	*L. dimidiatus* was always present but sometimes not cleaning mantas. Both adult and juvenile *T. lunare* were present and observed cleaning mantas
Fairytale reef	*T. lunare*	4.0 (SD±2.55) *n*=5 video sequences	Only adult *T. lunare* were reported from the analysed video sequences but other cleaner species were present
Boduhithi (Rasfari North)	*T. amblycephalum*	32.7 (SD±28.2) *n*=21 photo and video sequences	Range of 7-111 *T. amblycephalum* cleaning each manta
Table Thila	*T. amblycephalum* *T. lunare*	31.3 (SD±15.3) 0.58 (SD±0.65) *n*=24 photos	Range 10-67 *T. amblycephalum* cleaning each manta

Table 7.3 Results from analysis of photographs and video sequences examined to identify species and numbers of cleaners involved in cleaning a manta at various survey sites. Each sample was of a different manta.

Five species of wrasse were observed cleaning mantas in the Maldives. These were *Bodianus diana* (Lacepède, 1801), *Labroides dimidiatus* and *L. bicolor* Fowler & Bean, 1928, and *Thalassoma amblycephalum* and *T. lunare*. At each site cleaners were classified as primary (abundant and/or first to clean) or secondary (infrequently sighted cleaning/not always present) (Table 7.1).

The remora, *Echeneis naucrates* Linnaeus, 1758 was also observed in association with some mantas and its cleaner role is debated at the end of this section. Other reef fish are sometimes observed in the vicinity of mantas and rarely may clean mantas but there was insufficient evidence to confirm their role. The primary cleaner(s) species varied from one site to another and at different places at the same station and are described for each site in the relevant section.

Lankan Reef

On 18.7.2006 cleaner fish counts were conducted at Lankan Reef at the main cleaning station bommie (Figure 7.3). The results are reported in Table 7.2. The following species were observed cleaning mantas: *Thalassoma amblycephalum, Labroides dimidiatus, T. lunare,* and *Bodianus diana.* Initial phase *T. amblycephalum* (Figure 7.2) living in small schools (2-10) or larger congregations (max. size: 80), were the most common cleaner at Lankan. Cleaner fish congregated at four specific fixed aggregation locations (Areas 1-4) over the cleaning station (Figure 7.3). The mantas directed their swim pattern over the aggregation areas, see section 7.6.8.

Figure 7.2 Initial phase *Thalassoma amblycephalum*. This is the principal cleaner of *Manta alfredi* in the Maldives. Photos R. C. Anderson (Anderson 2005) with permission.

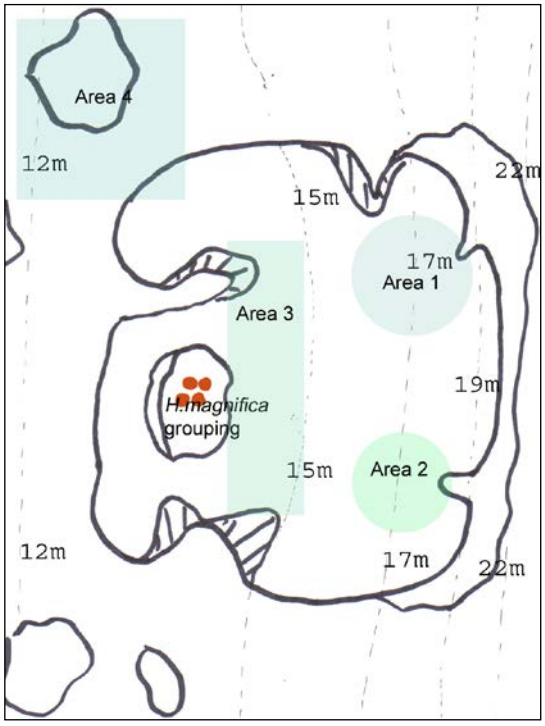


Figure 7.3 Diagram of Lankan cleaning station bommie to show main aggregations (areas) of cleaning fish (principally *Thalassoma amblycephalum*), areas 1-4. The site consists of a large *Porites* coral bommie about 15x12 m in size. The dotted lines represent the reef contours indicating that the block lies on a reef slope, top at about 12 m depth. The bommie lies between approximately 15 m and 22 m depth and is normally identified from the other similar large *Porites* bommies by the presence of a group of *Heteractis magnifica* anemones on the top at about 15 m. The reef slopes more gently beyond 22 m.

Sunlight thila

The cleaning stations at Sunlight thila consist of isolated coral blocks within a lagoon with specific blocks being favoured areas for being-cleaned by mantas (see Chapter 3, Figure 3.11), although cleaners were observed at the majority of blocks. At Sunlight the primary cleaners were *L. dimidiatus* (Figure 7.4), *T. amblycephalum* and *T. lunare* (Table 7.3). Analysis of photographs and video sequences showed 0-22 individuals of each cleaner species around a manta at any one time (and thus assessed to be cleaning the manta). In 12 out of the 16 samples, one or two of the species would be missing. Both adult and juvenile *T. lunare* were involved in manta cleaning and in some photographs it was difficult to determine whether initial phase *T. amblycephalum* or *T. lunare* were present and it is likely that the number of *T. amblycephalum* were overstated and the number of *T. lunare* were understated. Sunlight was the manta cleaning station most regularly visited by scuba divers from 1995 to 2003 because it provided the most regular manta sightings. The main cleaning station became “infested” with bluestriped fang blennies *Plagiotremus rhinorhynchos* (Bleeker, 1852) (Figure 7.5) in 2003. Aggressive mimicry (removing healthy scales and mucus) was described by Walker (1981) and specifically for *P. rhinorhynchos* towards other reef fish by Cheney and Cote (2007) and Cheney (2008). It has been reported that when reef fishes learn to recognise the mimic, they avoid the cleaning station altogether (Cheney 2008). The mimics were observed biting mantas and causing them to flee the cleaning station. This

period coincided with the increased use of Lankan reef by the same mantas which had previously been observed being cleaned at Sunlight.

Figure 7.4 *Labroides dimidiatus* the bluestreak cleaner wrasse and a primary cleaner at Sunlight thila (Photo by Brian Pickavance, from Age of Aquariums, www.aquahobby.com.au, with permission).

Figure 7.5 The cleaner-mimic bluestriped fang blenny *Plagiotremus rhinorhynchos* frequently sighted at Sunlight thila since 2003.

Fairytale Reef

Fairytale reef cleaning station, like those at Sunlight thila, consists of isolated coral blocks in a sandy lagoon area and was mainly used by mantas during September to November (the four surveys mantas were reported were in September and October and although visited throughout the year, mantas were not seen here before August or after November). The primary cleaners at Fairytale were *L. dimidiatus* and *T. lunare* with other species only being seen infrequently and thus considered secondary cleaners. During the five video sequences analysed, only *T. lunare* were reported (Table 7.3, Figure 7.6). A cleaning session typically involved only one or two individuals of each species but there were two or three species present at one time with the non-active cleaner fish remaining close to the reef. Water clarity and distance of observers from the mantas when cleaning prevented more quantitative study at this site as it was difficult to identify and count members of each species.

Figure 7.6 Manta 374 F5 (left) with two *T. lunare* cleaners. Manta 97 L64 (right) with seven *T. lunare* cleaners, indicated with arrows, observed at Fairytale on 22.10.2003.

Boduhithi, Table thila, Kalhahandi Huraa and Madivaru

These sites are different to the sites discussed so far (which consisted of a visually distinct *Porites* coral block bommie as the manta cleaning station). Other than Table thila, the reefs are all exposed ocean facing reefs and were inhabited by multiple aggregations of *T. amblycephalum* (estimate >>10 aggregations but not counted due to movements of cleaner fish) plus the other cleaner fish species as listed in Table 7.1. Aggregations of cleaners (particularly juvenile *T. amblycephalum* and *T. lunare*) were seen moving around the reef and there was not one specific favoured area used by the mantas, instead the mantas appeared to swim along the reef looking for cleaners and stop to be cleaned opportunistically.

On 9.1.2007 during surveys at Boduhithi (Rasfari North site), estimates for density of *T. amblycephalum* were difficult to make because both cleaners and host were continually moving, however analysis of 21 photos of mantas being cleaned had 7 to 111 *T. amblycephalum* on a single manta (as reported in Table 7.3).

At Table thila, cleaners were distributed all over a gently sloping reef in an area of around 20 m depth and extending to beyond 30 m depth, and also located at some shallower points along the coral reef slope. Video footage and photographs were analysed for numbers of cleaners attending to a single manta and reported in Table 7.3. Video sequences showed over 60 *T. amblycephalum* working on a single manta (see Figure 7.1), and many mantas being cleaned at the same time (Figure 7.7). A mean of ~17 mantas per survey were reported at this site (Table 7.7), suggesting 544 cleaners being involved in cleaning at one time (based on mean numbers). The aggregation areas

were distributed fairly evenly along the reef, but the areas visited by mantas to be cleaned changed depending on current direction, and tended to be in areas partially sheltered from current.

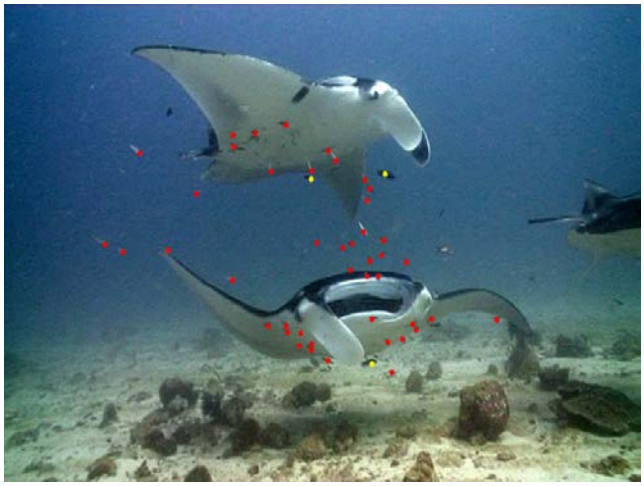


Figure 7.7 Analysed photograph of mantas being cleaned at Table thila on the limestone rock area at 20 m depth. There are no landmarks for the cleaners which seem to be evenly distributed over a wide area. The lower manta has approximately 30 cleaners and the upper manta has approximately 16 cleaners working on it.

The exposed reefs at Table Thila, Kalhahandi and Madivaru are subject to strong currents and surge during the SW (windward) monsoon. Other elasmobranch species were frequently seen being cleaned during both monsoons, including grey reef shark, white tip reef shark *Triaenodon obesus* (Rüppell 1837), and spotted eagle ray *Aetobatus narinari* (Euphrasen, 1790). These elasmobranchs visited the same reef areas visited by the mantas during the NE (leeward) monsoon. The cleaners were also observed cleaning other large (predatory) fish including humphead wrasse *Cheilinus undulatus* (Rüppell, 1837), Rainbow runner *Elagatis bipinnulata* (Quoy & Gaimand, 1825) and giant trevally *Caranx ignoblis* (Forsskål, 1775) as well as passing schools of fusiliers (*Caesio* spp. and *Pterocaesio* spp.) and reef fish.

At Kalhahandi there were aggregations of cleaner fish all over the plateau but mantas are only observed being-cleaned at the western margin in the area sheltered during strong outflow current. The cleaning stations consist of aggregations of *T. amblycephalum* and small colonies of *L. dimidiatus* but there are no distinctive physical structures. The *T. amblycephalum* aggregations tended to be located on the reef flat and crest, whilst the *L. dimidiatus* colonies tended to be slightly over the crest on the reef slope below 15 m. There is an area at the western extreme of the reef where *L. bicolor* was often seen, and was often occupied by giant trevally and grey reef sharks being-cleaned. A retrospective analysis of photos and video was hampered by the number of small fish in the water column around the cleaning stations which made it

impossible to estimate the number of cleaners with any accuracy (Figure 7.8) so no results are presented. From a random sample of 23 surveys made at the western margin during out-flow water movement between 2002 and 2006, mantas were sighted on 15 (65.2%) of occasions. The current experienced in the channel varied from zero to 1.5 m sec^{-1} (very strong) when mantas were seen.

Figure 7.8 A manta on the reef crest at the western margin of Kalhahandi Huraa site. It was difficult to identify cleaners amongst schools of orange anthias *Pseudanthias squamipinnis* (Peters, 1855) and red tooth triggerfish *Odonus niger*.

At Madivaru mantas were commonly observed at the extreme western section (length 300 m), swimming parallel to the reef crest at depths of 12-20 m apparently seeking to be cleaned. The area of reef visited by mantas varied from one survey to the next but the locations were not recorded. *T. amblycephalum* was the primary cleaner: adults/males and aggregations of initial phase/female fish were observed cleaning mantas. Initial phase *T. lunare* were also very common at this site and observed cleaning mantas. Aggregations of both *Thalassoma* spp. were seen along the reef crest whilst single or pairs of *L. dimidiatus* in fixed locations were common on the reef slope below 15 m depth. It was common to observe two or three single mantas or small groups of 2-3 mantas being cleaned at the same time at different areas 100-200 m apart, but only the total number of mantas per survey were recorded (and reported in Table 7.7). Quantitative analysis of cleaner numbers at this site was not undertaken as horizontal visibility and light conditions made counts very difficult.

Remoras

Live sharksucker *Echeneis naucrates* was the most common species of remora seen in the Maldives (Figure 7.10) and was often seen with mantas. Figure 7.9 shows parasitic lice on the dorsal surface of a male manta without remoras. The lice were approximately 1-2 cm in length and probably *Caligus* sp. It was hypothesised that older (and thus

larger) mantas would be more likely to have remoras in association as they would also be more likely to have collected parasites. To test this, the mean size (DW) of mantas with remoras was compared to the mean of the general population (all *M. alfredi* of known size, n = 1366) using a *t*- test. Mean size (DW) of mantas associated with remoras: 3.08 m (SD ± 0.79, n = 94) were not significantly different to that of the general population: mean 3.05 m (SD ± 0.55) (t = -0.30, P = 0.762).

Figure 7.9 Parasitic lice, order Siphonostomatoida (probably *Caligus* sp.) on the dorsal surface of a sub-adult male manta. (Photograph taken at Lankan, November 2007).

Figure 7.10 Large adult female manta with entourage of five remoras (*Echeneis naucrates).*

Survey Site	No. mantas with remoras	Total mantas this site	% with remoras
Sunlight thila	3	42	7.1
Lankan Reef	37	408	9.1
Fairytale Reef	0	8	0.0
Boduhithi	4	233	1.7
Table Thila	14	508	2.8
Kalhahandi Huraa	3	30	10.0
Madivaru	31	185	16.8

Table 7.4 Percentage of mantas reported sighted with remoras at main cleaning station survey sites.

Initial observations indicate that mantas with remoras do not carry lice (none observed) however too few examples of mantas with lice (and no remoras) had been recorded to make a statistical analysis. The percentage of mantas reported with remoras appears to vary between sites ($\chi^2 = 58.08$, $P < 0.005$, DF = 6) (Table 7.4). This is surprising as mantas are known to move between survey sites e.g. mantas seen at Table Thila may also be sighted at Madivaru etc. (see section 5.3.4). Further investigation as to why only some mantas are observed with remoras and the relationship of lice, remoras and mantas is required.

7.5 Conclusions from cleaner fish study

Five main species of fish were identified as cleaners of mantas at the cleaning stations surveyed. Based on these results, *T. amblycephalum* appears to be the most important cleaner of mantas in the Maldives (based on its frequency and abundance) despite not before being scientifically documented as a cleaner. It was present at all the main cleaning stations as either a primary or secondary cleaner, and occurs in large and numerous aggregations at certain sites e.g. Boduhithi and Table thila. *T. lunare* was present in both its adult and juvenile forms as a primary cleaner at over 50% of sites. Cheney (2008) and Oliver et al. (2011) mention the species in a cleaning context but do not indicate whether adults or juveniles were involved. All other references show pictures of adults and do not mention juveniles (e.g. species profile on www.fishbase.org). This appears to be the first documentation of juvenile *T. lunare* as an important cleaner

Each cleaning station was inhabited by 2-5 species of cleaner fish which lived in 1-10+ aggregations (depending on cleaning station) which consisted of 1-100+ cleaners of each species present. For example, the cleaner aggregation at Area 1 at Lankan reef consisted of over 60 individuals consisting of four species of cleaner, whilst the aggregations at Sunlight thila or Fairytale reef consists of up to 23 individuals from one to three species. The locations of aggregations were fixed or moving and even the numbers of fish within the aggregations varied from survey to survey on a single day based on the Lankan surveys. At Lankan reef there is one main bommie (the cleaning station) with up to four aggregations of cleaners. At Table thila, Boduhithi etc. there more than 20 cleaner fish aggregation areas and up to 111 individual cleaner fish were observed cleaning a single manta at once. These results indicate a wide range in the numbers of individual cleaners located at the cleaning stations visited by mantas.

The role of remoras is not fully understood although they appear to be a cleaner of mantas based on the absence of lice observed on mantas accompanied by remoras. The role of remoras is discussed further in the discussion section at the end of this Chapter.

7.6 Results and observation of manta being-cleaned studies

7.6.1 *Types of cleaning stations*

Category	Description	Examples
Single bommie	Single bommie (or small cluster of bommies), usually *Porites* spp., located at current convergence points or current eddy points near channel or on ocean facing reef. Typically 1-4 aggregations of cleaners per bommie.	Lankan reef, Maavaru, Helengeli thila, Fushifaru thila, Big thila, Mulidhoo corner, Kani, Kurali, Muli, Mudakan (10 sites)
Lagoon blocks	Isolated coral blocks in shallow lagoons with sand bottoms. 1-4 aggregations of cleaners per block.	Sunlight thila, Fairytale, Sandune, North channel, Desperation thila, Hanifaru, Dhiggiri (7 sites)
Outer reef flat	Area of reef crest or reef flat on ocean facing outer reef. >>10 aggregations of cleaners distributed over 100-500 m^2	Boduhithi Rasfari North, Madivaru, Kalhahandi huraa, Manadhoo, Alimathaa, Maa faru, Emas thila, Himendhoo thila (9 sites)
Area on thila	Aggregation of cleaner fish at specific area of thila, area may be visually distinctive. 10>4 aggregations of cleaners may be involved	Table thila, Boduhithi thila, Dhigu thila, Donfanu thila, Nelivaru, Iguraidhoo thila, Kottefaru thila, Ukulhas (8 sites)

Table 7.5 The four types of reef inhabited by the cleaners visited by mantas are categorised and all known (to the author) sites are listed within each category.

The type of reef inhabited by the cleaners (and which formed the cleaning station) varied from one site to another but the type may be categorised (Table 7.5). All sites known to the author were included. Single bommies and lagoon blocks were visually distinctive, rising out of the reef or sand bottomed lagoon. Often the blocks were located at current convergence points or at the entry to important channels. Cleaning stations located on outer reef flats and on sections of thilas were much less visually distinctive (less or no apparent separation from the rest of the reef). There was no apparent geographical distribution of a particular type of reef inhabited by cleaner fish and used as a cleaning station by mantas throughout the Maldives, i.e. one type being more prevalent in one atoll than another. An atoll usually contained a variety of types.

7.6.2 *Quantitative studies of manta behaviour at cleaning stations*

Number of mantas using cleaning station during day

The number of mantas observed being-cleaned during a full-day survey ranged from 19 to 93 mantas (Table 7.6). Some mantas (<5%) which were being cleaned could not be identified because they avoided the observers. Only one full-day observation was carried out at Madivaru due to the presence of other divers making data collection difficult. The high number of divers on the study day may also have deterred mantas from being cleaned.

Site	Date	Mantas individually identified
Lankan	18.7.2006	30
	26.7.2006	66
	31.7.2007	27
	8.8.2007	72
Boduhithi	9.1.2007	73
	9.2.2007	93
	24.2.2007	86
Madivaru	2.2.2007	19

Table 7.6 Number of mantas individually identified at cleaning station during full-day surveys

Time spent at cleaning station in 'cleaning activity'

The time spent by each manta at the cleaning station was recorded for four days at the Lankan survey site. The four sets of results were pooled and analysed to see how cleaning times were distributed. The time spent at the cleaning station ranged from 5 minutes (minimum unit recorded) to 260 minutes in total in a day (n = 193). The mean time was 35 minutes (SD ± 34.0) and median and modal times were both 20 minutes. Results are displayed in Figure 7.11 with details of the full day observations included in appendix C. The longer total times spent at the cleaning station often involved several visits. The longest continuous time recorded by a single manta at the cleaning station was 175 minutes.

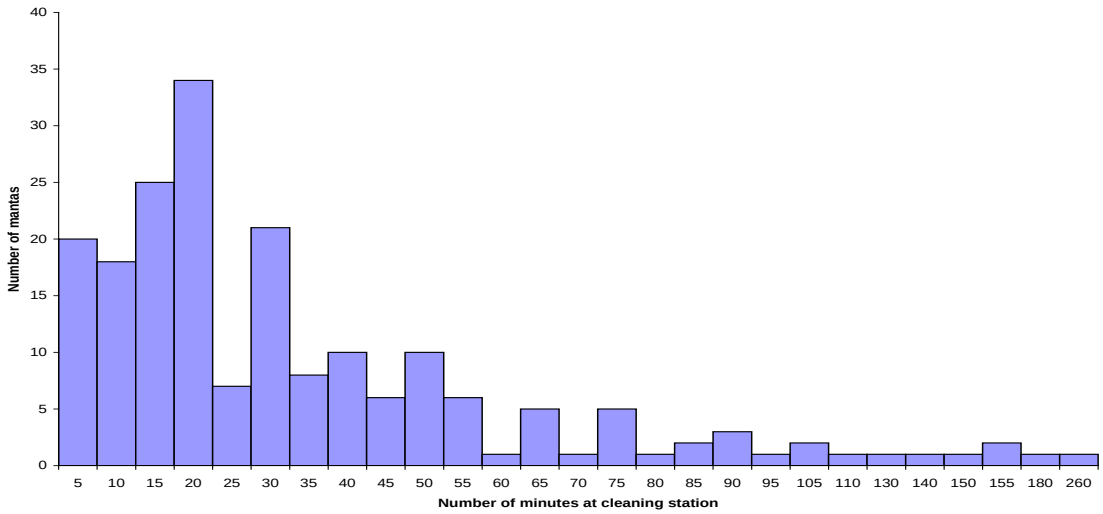


Figure 7.11 Frequency distribution of total time spent at cleaning station in a day by a sample of 193 mantas with times split into 5 minute classes. The mode was 20 minutes which was also the median time.

Number of mantas at cleaning station at one time

From zero to 16 identified mantas were recorded at the cleaning station at one time at Lankan, however there were times when more than this number were present but not all animals could be positively identified. When there were 6 or more mantas present at one time it was difficult to record exactly who was present as mantas would be cleaned and then swim away for periods of 30 seconds to several minutes. The frequency with which different numbers of mantas were seen (not necessarily all identified) at all sites per one hour survey (n = 325) in summarised in Figure 7.12 and Table 7.5. Note surveys when no mantas were identified were not recorded. The data were non-normally distributed, even when log-transformed.

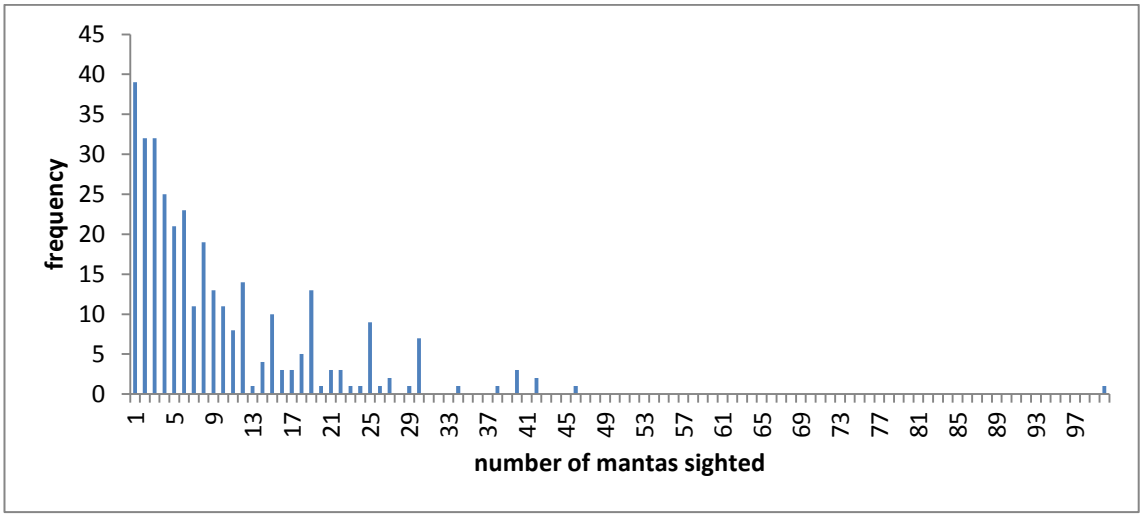


Figure 7.12 Frequency distribution of number of mantas seen at a cleaning station in a one hour survey. Observation of a single manta was most commonly reported. Zeros were not recorded.

Site	Size of site	Estimated area m²	N (surveys)	Mean number sighted ± SE	StDev	Median	Range
Madivaru	Large	600	63	5.13 ± 0.49	3.92	4	1-22
Lankan	Small	40	137	8.20 ± 0.60	7.04	6	1-34
Sunlight	Small	20	13	4.54 ± 0.84	3.02	3	1-11
Table Thila	Large	150	48	18.06 ± 2.43	16.82	15	1-100
Boduhithi	Large	400	44	14.16 ± 1.68	11.13	12	1-40
Kalhahandi	Large	400	13	5.08 ± 1.75	6.30	3	1-25
All sites	**n/a**	**n/a**	**325**	**9.486 ± 0.566**	**10.20**	**6**	**1-100**

Table 7.7 Number of mantas identified per survey (zeros excluded) at main cleaning station survey sites. "All sites" includes data from sites with small numbers of surveys.

As data pooled by site was non-normally distributed the site results were compared using Kruskal-Wallis test and then site combinations were investigated using Tukey-Kramer all pairwise comparison. The Kruskal-Wallis test (H= 50.92, DF = 5, $P < 0.001$, when adjusted for ties) suggests that the number of mantas seen at the main sites were significantly different, with the Tukey-Kramer comparison suggesting that the number of mantas sighted at both Table Thila and Boduhithi were significantly greater than when compared to all the other sites, but not significantly different between the two sites. To investigate whether there was any relationship in the number of manta seen and the size of the cleaning stations a correlation of number of mantas seen with estimated area was conducted however the result was not significant (Pearson correlation -0.080, P= 0.138). Whilst these results suggest that the number of mantas sighted varies significantly with site, they do not support the hypothesis that the number of mantas seen at a cleaning station may be related to its size.

7.6.3 *Distribution of manta activity at cleaning stations in daylight hours: Lankan Reef*

Graphs of numbers of mantas seen per five minute period (n = 498) during full day observation periods at Lankan Reef are shown in Figure 7.13 and results are summarised in Table 7.8. The presence of individual mantas at Lankan is listed in graphs in appendix C. These four days show the cleaning stations being used by mantas from 07:00 to past 17:00 on both rising and falling tides. The strength of current was not routinely recorded however it was noted that a strong current (>0.44 m.sec^{-1}) was experienced from 07:45 to 10:30 on 18.7.2006 and from 07:15 to 10:00 on 26.7.2006

(Figure 7.15 a, b) coinciding with periods of time when no mantas were observed. Mantas were observed during 35.1% of surveys before noon, and in 64.8% of surveys after noon however this result would have been influenced by the two periods of strong currents experienced in the morning during two surveys. Predicted tide height was added to the graphs (according to the tide charts produced by University of Hawaii at Honolulu). The tide rises and falls in a sinusoidal wave pattern however current velocity may not closely follow the tide pattern and the current experienced at any location may vary from that which might be predicted by the tide pattern (see section 3.3).

On 18.7.2006 (Figure 7.13 a) high tides were at 06:35 and 17:20 and the tidal range was only 0.33 m. No mantas were observed during the period after 07:45 to 10:40 when the tide was falling and the current experienced was strong. Mantas were reported much more regularly after 10:40 and until 17:00 apparently coinciding with the low to high tide period. On 26.7.2006 (Figure 7.13 b) low tide was at 07:26 and high tide was at 14:16 and the tidal range of 0.88 m was relatively large for the Maldives. No mantas were observed during the rising tide period after low tide (07:15 to 10:20) when the current experienced was strong. Greatest activity was around 12:30 and 15:00, the periods immediately before and after high tide. On 31.7.07 (Figure 7.13 c) low tide was at 07:00 and high at 13:46 and the tidal range was 0.89 m. The currents experienced were not as strong as had been predicted from the tidal range. There was low manta activity until around 12:15 but activity remained high throughout the afternoon, peaking around high tide. On 8.8.2007 (Figure 7.13 d) there were high tides at 09:43 and 17:11 and a low at 15:42 but the tidal range was only 0.12 m which is almost insignificant and there was manta activity throughout the day with peaks around 10:00 and 15:00 (high and low tide).

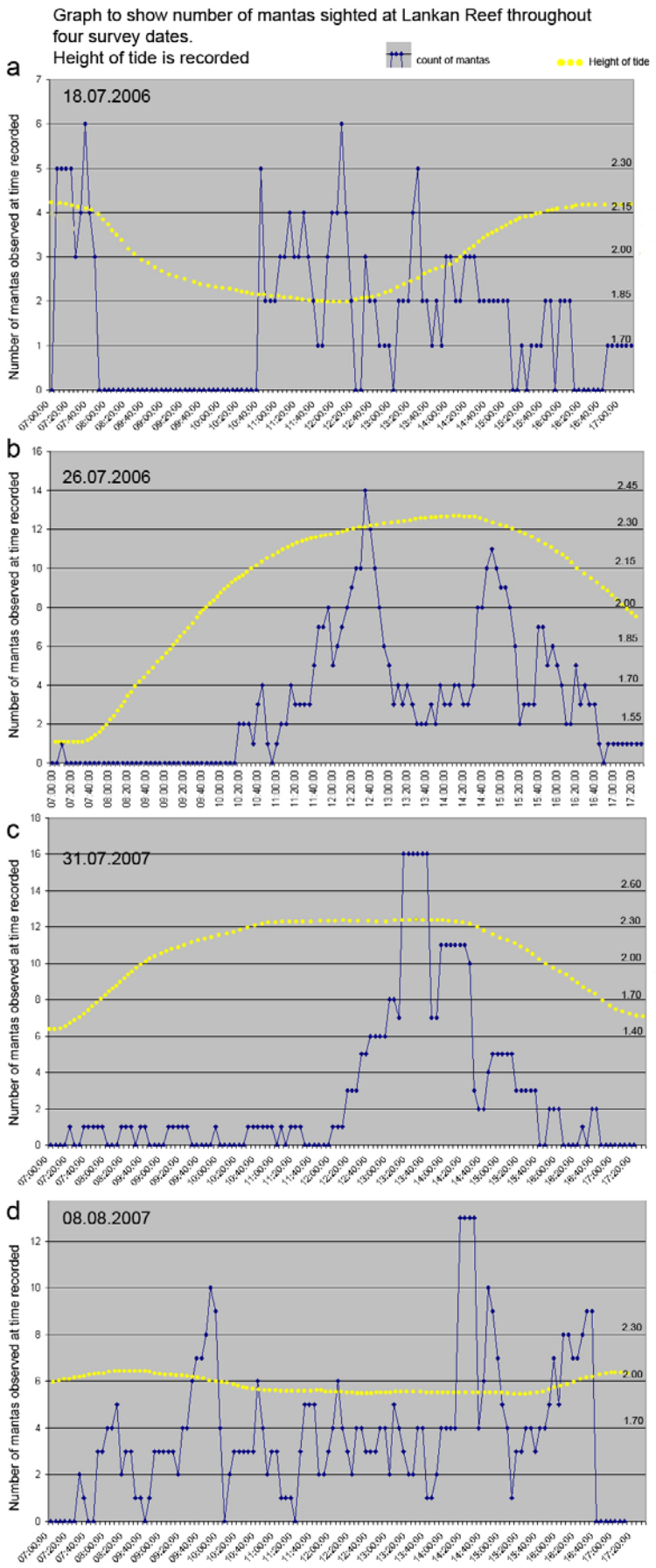


Figure 7.13 Graphs to show number of mantas at cleaning station at Lankan reef with time of day. The four graphs are for the four full days observations on dates indicated. The tidal height is also shown as the yellow dotted line

Day	N	Mean	SE Mean	StDev	Variance	Median	Total no. ind. mantas	Total time	Time/ manta
18/07/2006	123	1.520	0.145	1.611	2.596	1	30	935	31
26/07/2006	127	3.031	0.291	3.276	10.729	2	66	1925	29
31/07/2007	125	2.752	0.374	4.179	17.462	1	27	1720	64
08/08/2007	123	3.724	0.268	2.976	8.857	3	72	2290	32

Table 7.8 Summarised data from the four days of manta observation at Lankan cleaning station. Each unit represents the number of mantas seen in a five minute time segment on the relevant day. Data is not normally distributed for each day. Time per manta is the mean time each manta spent at Lankan that day.

The general results summarised in Table 7.8 were first investigated by comparing results between days. As the results were not normally distributed the means could not be compared. A Kruskal-Wallis test indicated that the numbers of mantas seen per 5 minutes on each days data were significantly different ($H = 43.17$, $DF = 3$, $P < 0.001$, when adjusted for ties). A Tukey-Kramer all pairwise comparison indicated that the numbers of mantas seen per 5 minute interval on 18.7.2006 were significantly different from other days but other combinations were not significantly different. On 18.7.2006 there was a falling tide in the morning and a rising tide in the afternoon compared to the other three days which had an opposite tidal pattern or negligible range. The smallest number of mantas was seen on 31.7.2007 but they individually spent more time cleaning than the similar number of mantas seen on 18.7.2006. These observations require further investigation.

The effect of current was analysed using Chi-squared test. There were 66 five-minute periods when the current strength was perceived strong (> 0.44 m.sec^{-1}) and only one period when mantas were seen. However there were 432 five-minute periods when the current was not strong and mantas were seen during 345 of these. Significantly more ($\chi^2 = 216.14$, $P < 0.001$, $DF = 3$) mantas were observed in low current conditions at Lankan.

Rayleigh tests to explain the effects of time of day and tide were made. The first analysis tested whether observations were uniformly distributed through arbitrary 12h

period of daylight hours (06:00-18:00) and gave a best estimate of the time when most sightings occurred based on a unimodal distribution over the day (mean vector for sightings). All days showed highly significant non-uniform distributions and showed mean vectors between 12:49h and 13:42h (Table 7.9). For tide, the average time between low and high tides (about 6.25 h) was the period investigated with a Rayleigh test to see if sightings were uniformly distributed over each (half) cycle between high or low tide. The results were not uniform, but not as significant in most cases as for time of day. Mean vectors showed low concordance ranging from 25 mins after H/L tide to 3:31h to 4:38 h after H/L tide, i.e. around mid-cycle. These results indicate that mantas may be seen at Lankan reef from before 07:00 until after 17:00 on both a rising and falling tide. There was significant variation between the numbers of mantas seen on different days. Most mantas were seen between 12:49 and 13:24, i.e. early afternoon based on a Rayleigh test. The effect of tide was less clear. A strong current appeared to have a very significant negative effect on the presence of mantas at Lankan and this observation requires further investigation, some suggestions for future experiments are outlined at the end of the Chapter.

1. Time of day	18/07/2006	26/07/2006	31/07/2007	08/08/2007	
$2nR^2$ statistic	35.8	363.4	426.6	86.47	All significant at P<<0.001
Mean vector	12:49 h	13:24 h	13:42 h	13:13 h	Best estimate of time when most mantas seen based on all observations

2. ~6.25h tidal cycle

$2nR^2$ statistic	29.9	22	265.9	93.6	All significant at P<<0.001. Distributed as X^2
Mean vector	00:25	03:31	04:38	04:01	after High/Low tide

Table 7.9 Results of Rayleigh test to investigate effect of time of day and state of tide on number of mantas. Number of mantas show highly significant non-uniform distributions throughout the day, with numbers peaking between 12:49 h and 13:42 h. The number of mantas seen was non-uniformly distributed with tide. The effect of tide Mean vectors showed less concordance ranging from 25 mins after H/L tide to 3:31h to 4:38 h after H/L tide.

Correlation between time spent by an individual manta in a day at Lankan Reef site with 1) the total number of mantas also identified cleaning during the same cleaning period and 2) the maximum numbers of mantas at the site at the same time were investigated.

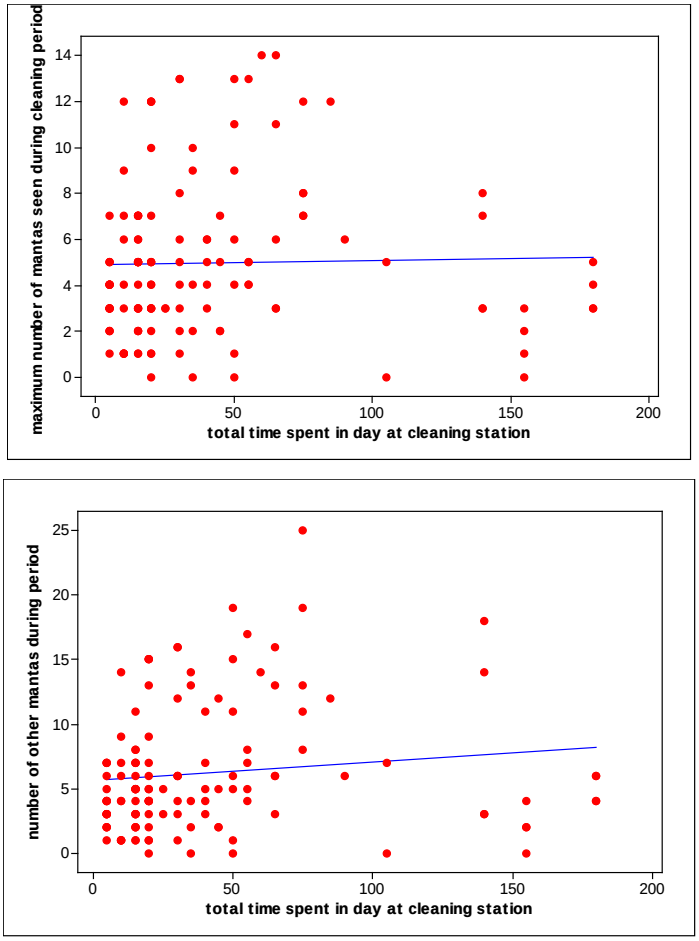


Figure 7.14 To investigate whether there was a relationship between time spent at a cleaning site in a day by an individual manta with number of mantas present at the same time, regression was used. In the top graph the total number of other mantas which were at the cleaning station at Lankan during the 132 periods of visit of 95 different mantas were analysed using regression. In the bottom graph the maximum numbers of mantas present at the same time during the period of visit of the study manta were analysed. Neither showed a significant relationship (F= 2.09, *P*= 0.126, DF=1; and F= 0.07, *P*= 0.791, DF=1; respectively).

The samples investigated were the 132 cleaning periods of 95 mantas recorded on 18.7.2006 and 26.7.2006. The cleaning periods lasted from 5 to 75 minutes each and the total time recorded at the cleaning station by an individual manta, in a day, ranged from 5 to 180 minutes. The regressions for both 1) and 2) were non-significant. (Regression for total number of mantas seen at cleaning station at same time vs. total time spent in day being-cleaned (Figure 7.14, top) F= 2.09, *P*= 0.126, DF=1; and regression for

maximum number of mantas seen at the same time vs. total time spent in day (Figure 7.14, bottom) F = 0.07, *P* = 0.791, DF = 1).

7.6.4 *Distribution of manta activity at cleaning stations in daylight hours: Boduhithi*

At Boduhithi, it was not possible to make precise recordings of arrival and departure of mantas. Mantas were commonly discovered already being-cleaned. Once a manta was identified, observers were encouraged to move to another area to identify the maximum number of mantas possible. For this study, mantas identified during each hour survey were compared instead of each five minutes as at Lankan. Figure 7.15 shows the number of mantas seen in one hour vs. time and the tide height. Peaks in manta numbers are seen early afternoon between 13:30 and 15:00 h, and 9.2.2007 had another peak at 11:00 h. If afternoon peak activity time is compared to high tide each day, then peak activity occurred at approximately 44, 67 and 82 minutes before high tide respectively for the three samples (mean 64.3 minutes). The additional 11:00 h peak on 9.2.2007 occurred after low tide that day. The tidal ranges (low to high) were only 0.45 m, 0.56 m and 0.62 m respectively on the three dates, which caused currents to be relatively mild.

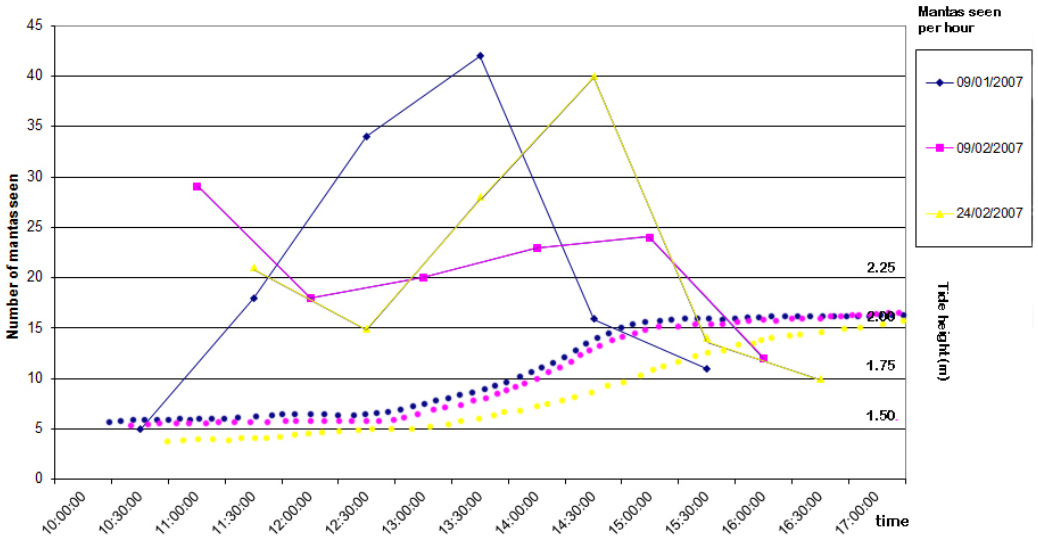


Figure 7.15 Graph of number of mantas identified during hour observation periods on the three day surveys at Boduhithi cleaning station. Height of tide is shown (non-connected circles) in same colour key for date as observation day.

The number of samples was too small from which to make conclusions, so all the state of tide was investigated for all 46 surveys conducted at Boduhithi when the time of day

was recorded. From these 46 surveys, mantas were recorded being-cleaned from before 07:20 h (mantas were already at the cleaning station at commencement of a 07:20 h survey) and until after 17:30 h. 50% of mantas were observed in counts during both morning and afternoon. 0-40 mantas were recorded per survey. No perceivable variations in current velocity were recorded during the surveys.

The number of mantas recorded on each survey per tide-state category (low, low-rising, rising-high, high, high-falling and falling-low) was analysed using one-way ANOVA and Tukey-Kramer all pairwise comparison. Results in each category were checked for normality using Anderson-Darling normality test, and were normally distributed ($AD \leq 0.428$, $P \geq 0.175$) except high tide ($AD = 1.214$, $P < 0.005$). One-way ANOVA showed that the number of mantas sighted during each category of tide did not vary significantly ($F = 2.08$. $P = 0.088$, $DF = 5$) and this was confirmed by the Tukey-Kramer comparisons which indicated no significant difference between pairs of results from any tide state. These results suggest that cleaning activity may occur throughout daylight hours at Boduhithi. The state of tide appears to have no significant effect on the number of mantas observed.

7.6.5 *Effect of state of tide and time of day on combined data*

All surveys where the time of survey was reported and mantas were reported ($n = 325$) were analysed to determine the tide state using AutoTide. Data binned by tide state were analysed to determine whether the number of mantas varied significantly with tide state using a Kruskal-Wallis test as data was nor normally distributed (Table 7.10). The result ($H = 4.46$, $DF = 5$, $P = 0.485$) supports the null hypothesis that there is no significant difference in the number of mantas seen with tide state.

The same data set was then analysed to determine time of day and binned into ten one-hour periods of when the surveys were performed. The summarised data are shown in Table 7.11. The data was again non-normally distributed so was analysed using a Kuskall-Wallis test to determine whether time of day had an effect on the number of mantas sighted. The result ($H = 24.92$, $DF = 9$, $P = 0.003$, when adjusted for ties) suggests that the number of mantas sighted varies significantly with the time of day. When the data was analysed using Tukey-Kramer all pairwise comparison test there was no significant difference between pairs of data sets (time bins), supporting the null hypothesis. Note: both of the above analyses are of sub-sets the data presented in Figure

7.12 which has a skew to the right, so the sub-sets shown Tables 7.10 and 7.11 also have skews to the right, i.e. the data were not normally distributed.

Tide State	N	Mean number of mantas sighted	Median	Range
Falling-Low	42	9.55 (SD±11.65)	4	1-42
High	70	9.63 (SD±13.15)	6	1-40
High-Falling	36	7.06 (SD±6.65)	5	1-40
Low	59	9.56 (SD±8.22)	8	1-100
Low-Rising	63	9.95 (SD±10.04)	6	1-25
Rising-High	55	10.24 (SD±8.85)	6	1-46
All	325	9.49 (SD±10.20)	6	1-100

Table 7.10 Summary of sightings data binned by tide state and analysed to determine whether tide state had an effect on number of mantas reported during each survey. Data was non-normally distributed so Kruskal-Wallis test was used to compare data bins. The result (H = 4.46, DF = 5, *P* = 0.485) suggests that tidal state has no significant effect on number of mantas sighted in each survey.

Time period of survey	N	Mean number of mantas sighted	Median	Range
07:00-07:59	76	9.36 (SD±9.18)	6	1-42
08:00-08:59	18	11.94 (SD±11.31)	7	2-46
09:00-09:59	4	4.00 (SD±5.35)	1.5	1-12
10:00-10:59	31	8.58 (SD±8.06)	6	1-30
11:00-11:59	99	10.69 (SD±12.56)	7	1-100
12:00-12:59	16	11.63 (SD±8.88)	8.5	1-26
13:00-13:59	13	14.77 (SD±10.21)	11	4-38
14:00-14:59	14	9.79 (SD±11.26)	4.5	1-40
15:00-15:59	41	6.05 (SD±6.66)	4	1-40
16:00-16:55	13	4.15 (SD±3.48)	3	1-40
All	325	9.49 (SD±10.20)	6	1-100

Table 7.11 Summary of sightings data binned by time of period of survey and analysed to determine whether time of day had an effect on number of mantas reported during each survey. Using a Kruskal-Wallis test to compare data bins, the result (H = 24.92, DF = 9, *P* = 0.003) suggests that the number of mantas sighted during a survey does vary significantly with time of day.

7.6.6 *Cleaning frequency study*

The assumptions used for each site in making the calculation of frequency of manta visits to the site are listed.

Lankan reef: mantas may be present for 11 daylight hours; the manta season at this site is eight months long (from mid-April to mid-December); mantas are not present during period of high current (see section 7.6.3), therefore assume absent for approximately 3 hours per tide cycle for 2 weeks per lunar cycle close to full and new moon; and there was a 85.1% success rate in reporting mantas per survey. **Sunlight thila**: same assumptions as Lankan reef except the success rate in reporting mantas per survey was 34.2%. **Boduhithi**: mantas may be present all daylight hours; season is 3.5 months (mid-December to end of March); mantas are not present during periods of strong currents and adverse surface conditions (50% of time); survey lengths approximately 1 hour; and there was a 70.1% expected success rate in reporting mantas per survey. **Table thila**[1]: mantas could be present 10 hours per day (07:00 to 17:00) during any current conditions for 4 months each year (mid-December to mid-April); surveys lengths 1 hour approx.; and success rate in reporting mantas is 81.3% per survey. **Kalhahandi**[1] : mantas present 10 hours per day (07:00 to 17:00) during outflow tides only (50%); season is 4 months long (mid-December to mid- April); but survey lengths were only 20 minutes. Note the 20.9% success rate in reporting mantas per survey (Table 5.1) was **all** surveys including inflow and out of season so 65.2% rate is used (NE monsoon only). **Madivaru**: mantas could be present 10 hours per day (07:00 to 17:00) during outflow tides only (75% of time- see explanation in section 3.3, Figure 3.5); the season is 4.5 months long (mid to late November to start of April); and surveys were 1 hour in duration with 69.6% success rate in reporting mantas.

Data presented in Figure 7.16 and Table 7.12 were obtained from analysis of data from Access database based on the first 50 mantas reported from each site. Table 7.13 summarises assumptions and results. The number and frequency of visits by a median/modal, average (mean) and most frequent manta visitor were estimated in order to describe the variety of visit frequency exhibited by this species at different sites in the Maldives. These results indicate that even mantas reported only once are likely to visit these known cleaning stations every couple of weeks, and near-daily visits were

[1] West Ari cleaning station sites (Table Thila, Kalhahandi and Madivaru) have 10 hour period when mantas are likely to be visiting cleaning stations based on author's observation.

predicted at all sites for the most frequent visitors as would be expected if a manta is cleaned daily.

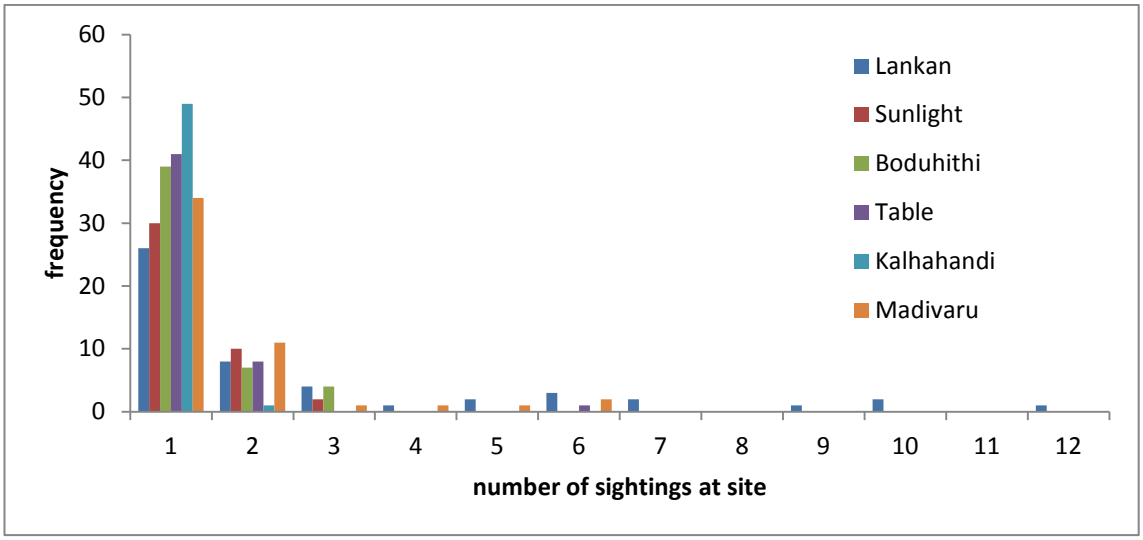


Figure 7.16 Frequency distribution of number of sightings of the first 50 mantas recorded at the site (or the entire data set for the site if <50).

Survey site	Sample size	Mean sightings per manta	Median/ Mode	Range	Total number mantas known to visit this site
Lankan reef	50	2.82 ± 2.83 SD	1	1-12	452
Sunlight thila	42	1.33 ± 0.57 SD	1	1-3	42
Boduhithi (RN)	50	1.30 ± 0.61 SD	1	1-3	320
Table thila	50	1.26 ± 0.78 SD	1	1-6	518
Kalhahandi huraa	30	1.03 ± 0.18 SD	1	1-2	30
Madivaru	50	1.60 ± 1.21 SD	1	1-6	188

Table 7.12 Summarised data (also shown in Figure 7.16) used to predict frequency of visit of mantas at main survey sites.

Survey site	No. hours per season when mantas may be present	No. surveys conducted	No. years (seasons) surveys conducted	Proportion of time per season spent in successful surveys	No. sightings of median sighted manta	No. sightings of mean sighted manta	No. sightings of maximum sighted manta	Predicted visit frequency per season of median sighted mantas	Predicted visit frequency per season of mean sighted mantas	Predicted visit frequency per season by max. sighted mantas
Lankan reef	2312	177	5	0.0153	1	2.820	12	13 times/ every 19 days	37 times/ every 6.6 days	157 times/ approx. every 1-2 days
Sunlight thila	2312	13	2	0.0028	1	1.333	3	178 times/ every 1.4 days	237 times/ approx. daily	534 times/ approx. twice daily
Boduhithi (RN)	639	47	3	0.0245	1	1.300	3	14 times/ ~every 8 days	18 times/ approx. every 6 days	41 times/ approx. every 2-3 days
Table thila	1217	52	4	0.0107	1	1.260	6	23 times/ every 5.2 days	29 times/ approx. every 4 days	140 times/ approx. daily
Kalhahandi huraa	608	14	4	0.0058	1	1.030	2	43 times/almost every 3 days	45 times/ almost every 3 days	87 times/ approx. every1-2 days
Madivaru	1027	71	6	0.0115	1	1.600	6	14 times/ every 9.5 days	23 times/ approx. every 6 days	87 times/ approx. every 1-2 days

Table 7.13 Table of predicted frequency of visit to main cleaning station sites by median (note: the result of 1 was also the mode in each category), average (mean) mantas and most frequent visitors based on reported sightings at each site. These results suggest that the most frequent visitors (maximum sighted) visit approximately daily at the majority of sites.

7.6.7 *Swimming speeds during cleaning activity*

Site	n	Mean (bpm) ± SD
Lankan reef	16	18.35 ± 2.22
Boduhithi	14	14.31 ± 2.84
Sunlight thila	15	16.79 ± 3.50
Table thila	13	14.62 ± 3.76
Combined	**58**	**16.13 ± 3.46**

Table 7.14 Mean swim speed (beats per minute) of mantas when being-cleaned, reported for four cleaning stations.

Swim speeds were measured as beats per minute (bpm) from sequences of mantas recorded cleaning at Lankan, Sunlight, Boduhithi and Table thila (Table 7.14). Each set of results was checked for normality using an Anderson-Darling test (*P* range 0.22-0.77). A one-way ANOVA analysis indicated there was significant variation between swim speeds at different sites (F = 5.58, *P* = 0.002, DF = 3) and when investigated with the Tukey-Kramer all pairwise comparison, swim speeds by mantas at Lankan were significantly faster than those at both Boduhithi and Table thila. The pooled set (n = 58, mean 16.13 bpm, SD ± 3.46) was checked for normality using an Anderson-Darling test (AD=0.39, *P* = 0.37) and compared to feeding speeds obtained from the study reported in Chapter 8 using two a sample *t*-test: being-cleaned combined (n = 58, mean 16.13 bpm, SD ± 3.46) vs. feeding combined (n = 58, mean 29.18 bpm, SD ± 5.54). This result indicates that mantas swim significantly more slowly when being-cleaned than when feeding (*t* = 15.22, *P* < 0.001, DF = 97).

7.6.8 *Observations of manta behaviour at cleaning stations*

Observations of manta cleaning movements at Lankan reef survey site

Sequences of movement and behaviour specific to cleaning activity were investigated from video tapes from 34 surveys conducted at Lankan. Videos of 70 approaching mantas were analysed. The number of different mantas cannot be identified from the video as the ventral markings used for individual identification were not evident, however repeat inclusion of individuals was likely to be low (<10%). Mantas unfurled their cephalic fins about 5 metres before arrival at the main cleaning station bommie at Lankan reef (Figure 7.3). This consistently observed behaviour establishes unfurling the cephalic fins to an “open” position as a precursor to cleaning (see section below for

analysis). Mantas with open cephalic fins were then observed orienting themselves to swim over the cleaners at Areas 1 or 2 from a southerly direction (Figure 7.16 shows an approach to Area 1 first) where they hovered above the cleaner fish to be cleaned for a few seconds before swimming away and making a small circuit of diameter less than 10 m and repeating the approach to Area 1 whilst keeping the cephalic fins open.

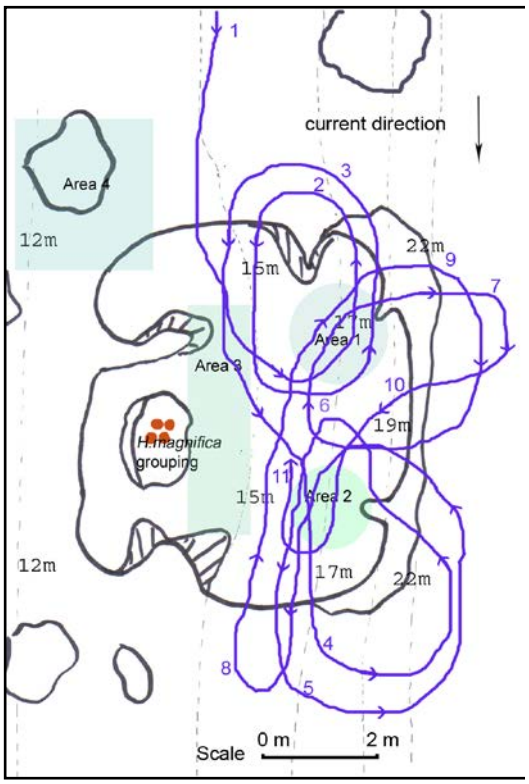


Figure 7.17 Diagram of Lankan reef main cleaning station bommie. The blue line indicates the swim pattern by manta 111 L78 observed 11.11.2009. The manta approached from the north (1) and swam in a southerly direction across the cleaning station. It then turned NE across Area 1 to be cleaned, rotated W (2) and then S, and made second approach to be cleaned. After being-cleaned it rotated W (3) and then S, but then continued across the block and rotated E (4) to approach and be cleaned over Area 2 and made a second circle around Area 2. This time it continued north (6) across Area 1 again to be cleaned and made a wide circuit (7). It then made a long southerly loop (8). It headed north again and was cleaned at Area 1 followed by wide loop east (9) then south (10) and north (11). This description represents around 4 minute's activity and the manta continued for around 12 minutes.

The current experienced at Lankan reef usually moves in a southerly direction and mantas faced into the current when swimming over cleaner fish aggregations. An example is shown in Figure 7.17 and the manta was observed using both Areas 1 and 2. Sometimes if Area 1 was occupied the other areas would be used, whilst at other times waiting mantas would make wider circuits to pace their arrival to Area 1 to coincide with another manta moving away. Figure 7.18 shows an example with a procession of three mantas swimming to the bommie from a southerly direction. In the photograph all mantas are maintaining their cephalic fins open but only manta (a) is actually above an area inhabited by cleaner fish (Area 1). The other mantas appear to be awaiting their turn to be cleaned at Area 1 but do not use Area 2. On other occasions when one group of mantas have occupied the cleaning station at Area 1 for several minutes, then other mantas did use the other cleaning areas. Unfortunately Areas 3 and 4 are close to where

observer divers wait and their presence of may inhibit mantas from using those areas (Figure 7.20). It appeared that disputes over access to Area 1 occurred between mantas on a frequent basis (estimate one dispute per survey) which were decided by agonistic displays (see section 9.3.2 for a detailed description of this behaviour) as shown in Figure 7.19.

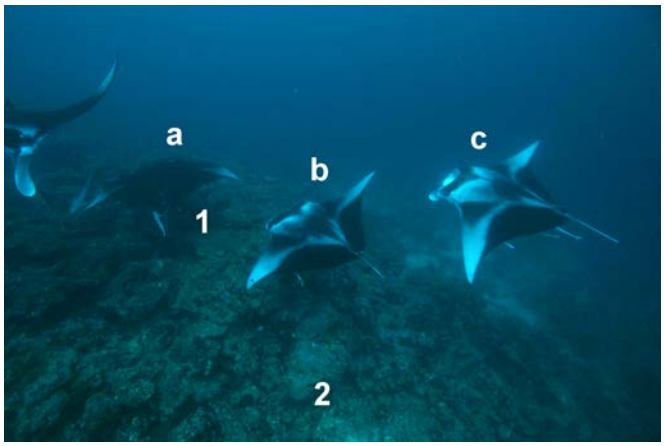


Figure 7.18 Mantas (a), (b) and (c) swam in from southerly direction parallel to reef towards Area 1 at Lankan cleaning station. In this photograph manta (a) appears to be being-cleaned at Area 1 whilst mantas (b) and **(**c) wait their turn but appear to ignore Area 2. All mantas have their cephalic fins open.

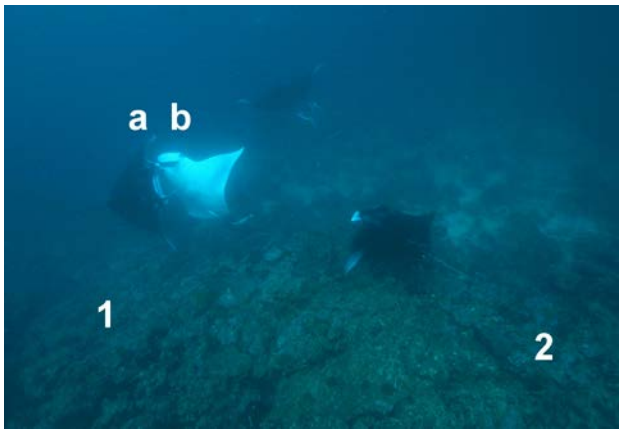


Figure 7.19 Two mantas, (a) and (b), appear to be performing an agonistic display to gain access to Area 1. Area 2 appears ignored.

Figure 7.20 A manta (centre) hovers over cleaning station Area 1. Approaching mantas in the background were forced to swim though the bubble curtain created by observing divers which may inhibit use of Areas 3 and 4 located on the periphery of the cleaning station. Photo: M. Shafraz Naeem with permission.

Actual cleaning of any manta by cleaner fish appeared to occur for a relatively small proportion of the time that the manta was at the cleaning station. Actual cleaning was punctuated by the manta making a short swim away from the cleaner fish, making a small circle or Figure of '8' (as shown in Figure 7.17) before repeating the orientation towards Area 1. Throughout this time the cephalic fins were maintained loosely open. As noted in section 7.6.2, the mean time spent around the cleaning station was 35 minutes, and median and modal time was 20 minutes. These times represent the number of minutes spent in close vicinity of the cleaning station but not of the manta ray actually being cleaned by cleaner fish. The time spent hovering over the areas of cleaner fish and actually being cleaned is estimated at a minimum of 20% of time spent in the vicinity of the cleaning station. It was difficult to determine the precise period of time when the manta was being cleaned as cleaner fish sometimes swam alongside the manta for short period, but did not appear to be actually cleaning the manta. Also, sometimes mantas swam though aggregations of cleaners and at other times they hovered amongst them. Thus, the precise start and end of the time spent actually being-cleaned was difficult to determine. If the time spent amongst cleaners is time actually being-cleaned then the time mantas are cleaned for may be as little as 4 (based on median/mode) or 7 (mean) minutes per day. This behaviour of swimming over areas of cleaner fish and making circles, or figures of '8', was observed at all survey sites but was moderated by local current conditions.

Observations of cephalic fin positions and posturing

There are three cephalic positions adopted by mantas in different behavioural situations: 'furled', 'open-feeding' and 'open-cleaning'.

Furled cephalic fins position

When the cephalic fins are tightly rolled up, they are termed 'furled'. This position was observed when mantas were swimming and neither feeding nor cleaning. This position was first described by Bigelow and Schroeder (1953) and was described as allowing for good streamlining whilst swimming quickly (Figure 7.21). I was unable to quantify the mean swimming speed on non-cleaning and non-feeding Alfred mantas for comparison. Mantas observed swimming towards (distance >5 m) and away from cleaning stations had their cephalic fins in this position.

Figure 7.21 Furled cephalic fins were observed on mantas which were neither feeding nor being-cleaned. The position would allow good streamlining for swimming quickly.

'Open-feeding' position

With the 'open-feeding' position, cephalic fins were locked into an exaggerated 'O' shape (Figure 7.22) and the mouth was opened to channel plankton to the gill rakers. Feeding and cleaning behaviours were distinct and no feeding behaviour was ever observed by a manta whilst being cleaned. Feeding behaviour is discussed in Chapter 8.

Figure 7.22 The open-feeding position. The cephalic fins are unfurled and create an exaggerated 'O' shape. Note that the fins meet below the mouth. The mouth and buccal cavity are well expanded. The cephalic fins help direct plankton into the open mouth.

'Open-cleaning' position

Figure 7.23 The open-cleaning cephalic fins position. The cephalic fins hang loosely open and the mouth remains shut.

In the 'open-cleaning' position (Figure 7.23), the cephalic fins hung loosely open, but the ends did not meet and appeared to be held in a much more relaxed manner than in the open-feeding position. The mouth was normally only partially open though it might

also be fully opened whilst being cleaned to allow access of cleaning fish into the buccal cavity (see Figures 7.7, 7.20 and 7.25).

Figure 7.24 The mouths of mantas being cleaned remained partially open to allow water flow over the gills whilst swimming slowly to maintain position above the cleaning station. Frequently, the mouth is slightly open on one side only. Photo: M. Shafraz Naeem with permission.

The open-cleaning position was adopted as the manta approached the cleaning station and then maintained throughout the period of cleaning. If the mouth was fully opened to allow access by cleaners the cephalic fins remained loosely open (Figure 7.25), not locked into an 'O' shape as seen when feeding. Typically the manta hovered above the cleaner fish, swimming into a current (so that the gills might be oxygenated) with the mouth opened a little on either the right or left side (see Figure 7.23). This is the first description of this pose.

Figure 7.25 Photograph of manta being cleaned which has opened its mouth and expanded its buccal cavity to allow access by the cleaners. This only occurs once cleaning has started. The cephalic fins remain loose, and do not form a tight 'O' shape.

22 random video segments of mantas being cleaned (180-600 seconds in length) were analysed to identify a signal-response by the cleaners to mantas. Video sequences were

specifically reviewed for cephalic fin positioning. The following observations were made:

1) Video sequences of mantas approaching cleaning stations always showed mantas with cephalic fins in open-cleaning position approximately 5 metres (estimated distance) before arrival at cleaning station.

2) Of 80 mantas arriving at a cleaning station, or already cleaning, 75 had cephalic fins in the open-cleaning position, three had one open and one furled, and two had fins in the furled position. All five of the exceptions (one or both fins furled) were of mantas already established cleaning. The two examples where the manta had fins in the furled position showed the mantas then swimming away and the fins may have been furled because the videographer frightened them. A Chi-square test was used to determine whether this behaviour (holding the cephalic fins in the open-cleaning position when arriving at a cleaning station and being cleaned) was significant whilst in the vicinity of the cleaner station. If this is a random behaviour the fins might be randomly open or closed i.e. 50 % of mantas would have them open. The Chi-square test showed that there is a significant bias to mantas having cephalic fins in open-cleaning position whilst at cleaning stations: $\chi^2 = 61.25$, $P < 0.001$, DF= 1.

3) Some mantas presenting themselves to cleaners with cephalic fins in the open-cleaning position were apparently ignored (3 were certainly ignored, and in two other cases it was difficult to determine whether the fish in the vicinity were cleaners or not). Thus making the posture did not ensure cleaning by cleaners.

4) Mantas with partially furled cephalic fins whilst being cleaned may have been disturbed by the observer (partially closed eye looking at photographer, Figure 7.26).

Figure 7.26 Manta at cleaning station with furling cephalic fins. In left photograph fins are still open, but starting to furl shut and eye is closing. In right photo fins are furled. The manta was cleaned for a few more seconds and then swam away.

Mantas were observed cleaning in moderate to strong current (0.5-1 $m.s^{-1}$) at Table thila. The changes between the furled and open-cleaning cephalic fin positions were obvious in these conditions because the mantas were not circling around. Instead, mantas adjusted their swimming speeds to hover over the cleaning station. Schools of over 20 mantas were observed hovering, facing into the current above the cleaning stations, and taking turns to break away from the group and hover over a cleaner fish aggregation. The mantas maintained their cephalic fins furled whilst not being cleaned. Mantas attempting to be cleaned opened their cephalic fins and swam closer to the reef. Sometimes cleaning would occur and sometimes it would not. In one video sequence of mantas at the cleaning station in particularly strong current, the cleaner fish remained close to the reef for most of the sequence (≈90% time) so minimal cleaning could have occurred. Lack of cleaning may have been due to the strength of the current making it difficult for the cleaners to approach the manta. There were no observations of cleaners approaching mantas with furled cephalic fins even when mantas were close to cleaner aggregations.

Observations of behaviour modification induced by diver presence

The most commonly observed behaviour by mantas induced by the presence of divers was that of swimming through the exhaled bubble streams of the scuba divers. This behaviour was termed ‘bubbling’ and was observed at all cleaning stations visited and performed by the majority of frequently sighted mantas. Mantas may swim over the head of an observing diver either to access a cleaning station or apparently, by intent. During most (estimate >90%) surveys, a manta was observed swimming very slowly over the head of a diver, and apparently, waiting for the diver to exhale. Frequently, several mantas were observed performing this behaviour, several times each, during a single survey. Occasionally the manta was observed to close its cephalic fins and swim away (apparently distressed by the interaction), but generally the majority of mantas which performed this behaviour appeared to be seeking out the exhaled bubble stream and hovered to maximise exposure (Figure 7.27).

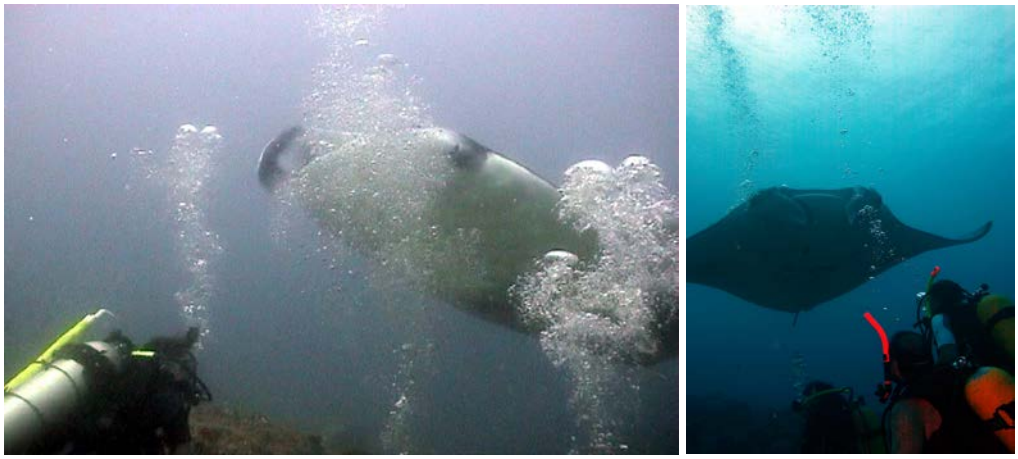


Figure 7.27 A large female manta known as ‘Bubbles’(116 L83) has become habituated to scuba divers and appears attracted to exhaled bubble streams, hovering in them to maximise exposure.

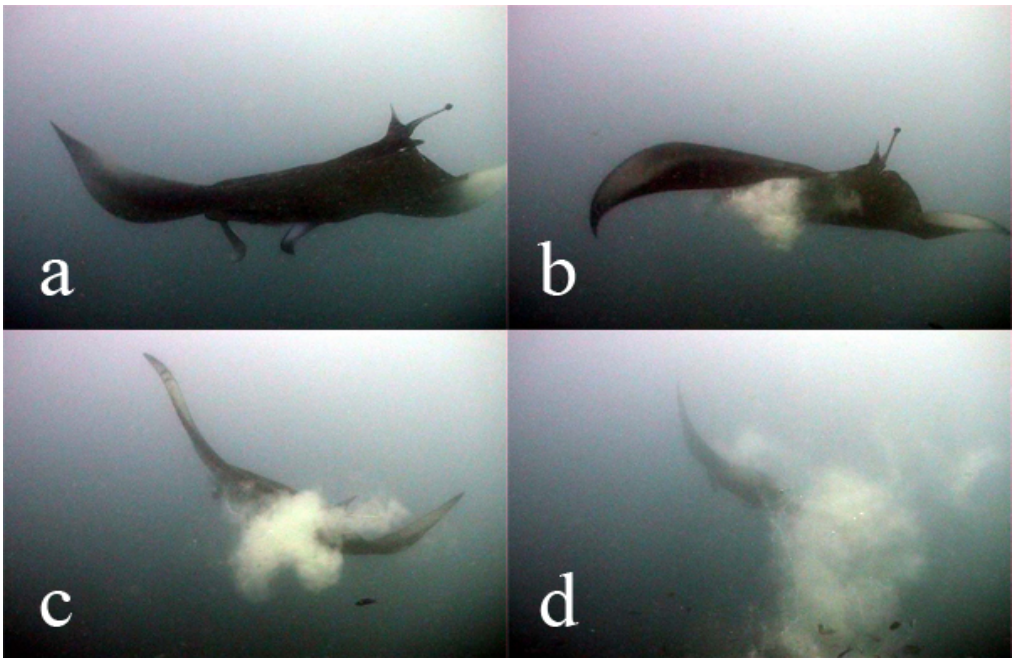


Figure 7.28 Frames from video sequence from Lankan reef. In (a), a manta which has swum through a diver’s exhaled bubble stream, swam away from the cleaning station. In (b) swallowed air was squashed out of the left gill-slits. In (c) the expelled air continued to expand and (d), the manta swam away.

Behaviour I term ‘advanced bubbling’ was also observed and recorded at most sites, but was performed by a much smaller number of mantas (estimate 20 to 30 individuals). In ‘advanced bubbling’ the manta swam through the bubble stream with mouth open and swallowed some exhaled bubbles which were then ejected through the gill-slits. When swallowing after feeding, mantas use the transverse curtain on the roof of mouth as a valve to prevent egress of water from the mouth when the walls of the pharynx are

contracted (Bigelow and Schroeder 1953). With the gills closed, the movement of the pharynx sucks plankton captured in the fine mesh of the gills towards the stomach. In the ‘advanced bubbling’ technique, the mouth was closed and the pressure caused by the contracting pharynx would force water and air out through the gill-slits (Figure 7.28). In Figure 7.29 the manta hovered motionless as it squeezed out the air it has swallowed, and there appeared to be much less air than the example in Figure 7.28.

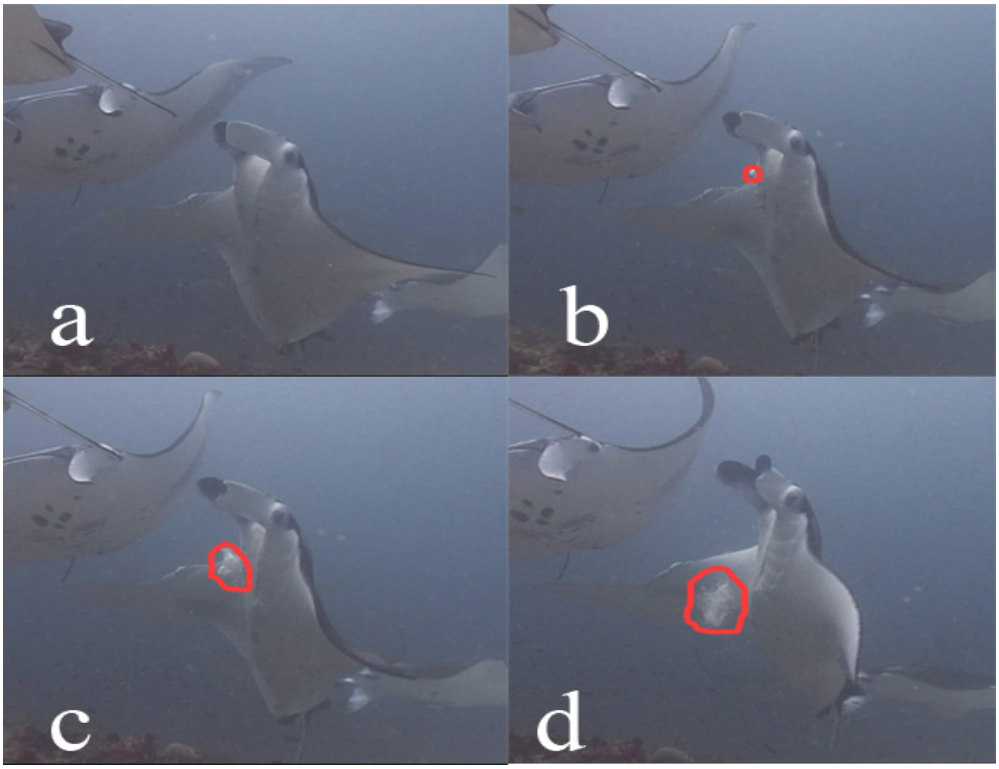


Figure 7.29 Frames from video sequence from Table thila. (a) A manta hovered vertically (an unusual posture) having swallowed some air (exhaled by a scuba diver). In (b) the air is seen coming out of the upper right gill-slit. The air was under pressure and can be observed expanding in scenes (c) and (d) but the manta continued to hover vertically.

7.7 Discussion

7.7.1 *Cleaners of mantas*

Section 7.5 indicated there were five species of wrasse which act as cleaners of mantas in the Maldives, and that these wrasses lived in groups of 2 to 60+ animals with the groups containing 1-3 species. The actual numbers of cleaners involved in cleaning mantas varied significantly with site. Few (<5) *Labroides dimidiatus* or *Thalassoma lunare* were involved in cleaning a single manta at Sunlight thila or Fairytale, whilst up to 117 *T. amblycephalum* were involved in cleaning a manta at Boduhithi. A high density of cleaners may be the specific attraction for mantas at certain sites but there

does not appear to be a direct correlation between sizes of sites (and therefore number of cleaning areas available) and number of mantas using the site. At Lankan there were at least 15 areas distributed along 300 m of reef which were known cleaning stations to various species of fishes and contain similar mixes of cleaner fish to those reported at the main bommie. Despite this apparent wide choice of cleaning areas, mantas consistently chose the cleaning station consisting of Areas 1-4 on the one bommie (Figure 7.3). Additionally, of the four aggregations of cleaners on the cleaning station at Lankan, the mantas prioritised their swim patterns over Area 1. This behaviour is indicative that there are "hotspots" of preferred cleaner aggregations for mantas. This appears to be an example of a client fish choosing cleaners which are particularly effective as described by Bshary and Schaeffer (2002), and discussed further in section 7.7.3.

That other fish species are observed being cleaned at the same locations as the mantas (but in their absence) suggests that the cleaners are not dependant on the mantas as a food source but only clean them in priority to other species due to their greater size. This observation is in accord with Grutter et al. (2005) who found that cleaner fish choose larger clients in empirical tests. A greater size is likely to offer a larger food source. However, Arnal et al. (2001) showed that although fish with high ectoparasite loads visit cleaning stations most frequently, cleaners did not clean high parasite load clients first. Predatory clients are prioritised by cleaners (Bshary 2002; Soares et al. 2007), so it may be a combination of larger size and perceived predatory status which makes cleaners prioritise cleaning of mantas. Mantas are known to eat small fishes (Bertolini 1933). During the night dive reported in Chapter 8, the fish predated by the mantas were juvenile mackerels (Scombridae), and of similar size to the smaller cleaner fish (4-8 cm length), thus cleaner fish may consider mantas a potential predator.

The study of cleaner fish at Lankan reef indicated that numbers of cleaners varied by as much as 50% from one survey to the next, even during a day, suggesting that the population of cleaners is not resident, but may be attracted to the area by the presence of a preferred client as proposed by Bshary and Schaeffer (2002) and Soares et al. (2007). When the site was an open expanse of reef (i.e. Boduhithi, Madivaru, and Table thila) the cleaners were observed moving around, following the mantas. These examples appear to show cleaners being attracted to a particular client and travelling around a site to have access to a client and appear to show cleaners congregating at the food source as

hypothesised in the references above. If both cleaners and mantas move to a particular area, the “hotspot” may be formed by a combination of mantas seeking out a specific area where the cleaners congregate, and the cleaners (which congregate at that spot) are those which prefer to clean mantas. Mantas cannot apparently tell the difference between cleaners and false cleaners (Stummer et al. 2004; Lettieri and Streelman 2010) based on the observations at Sunlight thila where mantas were observed at the same time as *Plagiotremus rhinorhynchos*. The latter mimics *L. dimidiatus* but bites the mantas and eventually drove them away from the Sunlight thila site by repetitive attacks over several months.

Members of the Echeneidae were assumed to be cleaners of external parasites on free-swimming sharks (Randall 1958; Feder 1966; Lewis 1967) and are known to consume the ectoparasitic copepod *Nesipus* sp.(Vaske 1995). At least 17 species of *Caligus* sea lice are known to infest elasmobranchs (Tang and Newbound 2004) including four ray species. There is only one mention of a *Caligus* sp. on a manta and that was of 35 *Caligus aiiuncus* Wilson 1905 collected from an individual manta (species unidentified, but not likely to be *M. alfredi*) in Panama (Bond et al. 1944). *Caligus* sp. were also reported on other epipelagic fish from tropical waters (Hayward et al. 2006). The presence of remoras on mantas is not fully understood. *Caligus* sp. lice are observed on some mantas which do not have remoras (*E. naucrates*) in attendance, but not on mantas with remoras. It is possible that the remoras act as cleaners of the mantas by removing larger parasites (Smith 1997) as well as being parasites, clinging onto the manta and reducing hydro-dynamicity of the host, in order to be transported to feeding areas of zooplankton which is also food to the remoras (R. Rubin, pers. comm.). A study to examine the relationship between lice and remoras can easily be carried out by routinely recording the presence of lice as well as remoras in future surveys. Personal observation is that once a manta is associated with remoras it continues to be associated with remoras and this hypothesis can also be examined by the routine recording of the presence of remoras. It was anticipated that remoras would be more attracted to larger mantas but mantas with remoras are not significantly larger than the general population of mantas so this hypothesis is not supported. The presence of remoras on mantas may be localised as mantas at Madivaru were more likely to have remoras than other sites (Table thila, Boduhithi). The variety in proportion of mantas with remoras at different sites warrants further investigation.

7.7.2 *Use of cleaning stations by Alfred mantas in the Maldives*

The time spent in the close vicinity of a cleaning station in single day varied between individual mantas. The mode/median time was 20 minutes and mean time was 35 (SD ± 34.0) minutes per day yet some individuals spent up to 260 minutes. It was noted that the time spent actually being cleaned may be as little as 20% of the time spent in the vicinity of the cleaning station. The rest of the time was spent in making circles away from the cleaner aggregations, waiting/queuing for access to cleaner fish, and in social interaction with other mantas. This pattern of clean - swim away - clean was also reported by O'Shea et al. (2010) for mantas (reported as *M. birostris* but probably *M. alfredi* from location and photographs) at Osprey Reef, Coral Sea, Australia. Significantly more females were observed than males (females-males sex ratio: $\chi^2 = 109.2$, $P < 0.001$, DF = 1) and the females observed were larger than males ($t = -22.89$, $P < 0.001$). The cleaning activity is occasionally interspersed by dominance displays between mantas, as in Figure 7.1, and this may be why more (larger) females are seen at cleaning stations (see Chapter 9). The dominance displays may be performed due to competition for access to cleaning stations and not the reason why they visit the cleaning station.

O'Shea at al. (2010) indicated a mean cleaning time of 31 minutes (maximum 300 minutes) which is similar to these results but they commented that cleaning was almost a continuous process during this time. In the Australian study 84% of mantas being cleaned were solitary. In this study only 39 surveys reported a solitary manta. 39 out of 2445 recorded sightings is 1.6%, i.e. the majority of mantas were sighted in the presence of other mantas. Competition may also have reduced the amount of time that mantas actually spent cleaning. The investigation of total time spent cleaning in a day with numbers of mantas present showed there was no significant correlation, which was a little surprising. The conclusion from this result would be that individual mantas spend widely varying amounts of time at a particular cleaning station and number of mantas present cannot be used to predict how long an individual may be cleaned for.

The number of individual mantas identified in a day long survey varied substantially (19 to 97) and up to 100 mantas were reported present at a survey site at any one time. The mean number of mantas attending a cleaning station per one hour survey was 9.49 (± 10.20 SD) although the mode was 1 and the median 6. The number varied significantly with site and there were no useful predictors identified. Instead, numbers are likely to be

influenced by local conditions at a specific site. A positive relationship between sizes of mantas and the number of times they were sighted was identified. This may be due to larger (likely older) mantas requiring more frequent cleaning, or to their size conveying greater social status deterring smaller mantas from the cleaning station. It was hypothesised that smaller sites like Lankan or Sunlight may limit access to cleaners whilst larger sites with many cleaning aggregation areas like Table Thila and Boduhithi would be unlimited but instead the lack of correlation supports suggest that certain cleaning stations are simply more attractive to clients, and this may be due to the behaviour of the cleaners as proposed in previous research (Bshary and Grutter 2002; Bshary and Schaeffer 2002).

The influence of tides on elasmobranch cleaning activity was reported by O'Shea et al. (2010) where likely *M. alfredi* were seen in larger numbers during ebb tides. The combined data from all sites suggested that there was no significant effect in number of mantas seen at cleaning stations during different tide states in the Maldives i.e. there was no overall trend. Some local variations were reported e.g. at Boduhithi more mantas were observed on a low to rising tide with activity peaking around one hour before high tide during the consecutive survey study days, but when all surveys conducted at Boduhithi were analysed the number of mantas seen during surveys at different tide states did not vary significantly. As only one cleaning station was reported by O'Shea et al. (2010) it is unclear whether this observation has any generality. It appears current may have a significant effect in the Maldives on manta cleaning activity. At Lankan, more mantas were sighted when a low current velocity was experienced (<0.44 m.s^{-1}), and although not specifically measured, Boduhithi is only surveyed when the current velocity is low (section 3.4.1) as mantas are known to be absent when the current is strong (the cleaning stations may be surveyed from the surface to detect the presence of mantas). The effect of current should be investigated further as it appears that it may be a more useful predictor of manta presence at cleaning stations than tide state.

Dewar et al. (2008) reported mantas (reported as *M. birostris* but now known to be *M. alfredi*) detected around Komodo coming into shallow reefs to feed and be cleaned during daylight hours: some mantas visited the same reef for many consecutive days. Using assumptions to account for conditions when mantas were likely to be observed, known season lengths for the various cleaning stations, and results from observation surveys, it was possible to predict the frequency of visit to a cleaning station by manta.

The results predicted that even mantas only ever sighted once (so far) were likely to visit the cleaning station over a period 2-3 of weeks. The most frequent visitors were predicted to visit near-daily at all sites. This pattern was similar to that observed around Komodo. It is likely that adult *M. alfredi* make a daily visit to a cleaning station, as reported in reef fishes by Feder (1966). They are large pelagic animals and they probably have a number of cleaning stations within their daily range. They may visit a number of cleaning stations, some which will be unknown to observers, explaining the spread in predicted frequencies for different cleaning stations and for different mantas (and the results obtained at Hanifaru in Chapter 6 during the consecutive day surveys). If individual *M. alfredi* visit a cleaning station daily, as with other fish, the frequency of visits is unlikely to be driven by parasite load (Arnal et al. 2001). The reason larger females are seen more frequently may be due to competition between mantas. There may be habituation of these larger females to divers and consequently, they will be more likely to be seen at cleaning stations which are frequently visited by divers.

7.7.3 *Manta behaviour at cleaning stations*

Table 7.5 lists the four categories of cleaning station type. Two of the categories could be considered visually distinctive and mantas might locate cleaning stations by searching for a physical marker which may be the particularly large or isolated bommie or visual contrast between white sand and dark reef in a channel. A different type of marker for a site would be an area where currents converge. These markers would need to be distinct enough that the location is remembered for subsequent visits. Sharks are known to possess excellent sensory systems (Hueter et al. 2004) which are important for location and orientation (Montgomery and Walker 2001) to a variety of clues, from water movement (Maruska 2001; Peach 2001) to visual stimuli (Gruber 1977; Hueter 1990). Mantas are known to have good eyesight (Ari and Correia 2008) and are likely to use other elasmobranch sensory systems so distinctive reef formations and variations in currents at current convergence points are likely to act as markers for these locations. Once mantas are established at a cleaning station, their presence may act as markers for other arriving mantas which then imitate the behaviour of the established mantas. The location and selection of cleaning locations requires further investigation.

Many fish and elasmobranchs adopt specific colour changes or unusual poses prior to cleaning which have been interpreted as a signal of their intent to be cleaned (Feder 1966; Keyes 1982; Cote 2000). It was to be expected that mantas would also pose when

signalling their intent to be cleaned. The repeated observations of cephalic fins being unfurled and then maintained in the open-cleaning position at cleaning stations is indicative that this is a posture maintained by mantas requiring to be cleaned. That no manta was cleaned when cephalic fins were fully furled suggests that cleaner fish recognise the posture as a signal by the client manta. The mean swim speed of 16.13 (± SD 3.46) bpm appears diagnostic for cleaning and can be used to separate cleaning and feeding activity if the cephalic fins position is in any way unclear. The effect of current velocity on cleaning activity swim speed should be investigated in future studies as it was not measured here. Current was indicated as an important predictor on the presence of mantas at Lankan (mantas were absent during periods of strong current), yet mantas were observed being-cleaned at other locations (particularly Table Thila) in relatively strong current.

The presence of divers is likely to affect a manta's choice of cleaning site. Mantas are often observed swimming away as divers approach. Martin (2007) reported that avoidance was an elasmobranchs' behaviour of choice when a competitor made a competitive display to have access to a resource (e.g. food, territory), to avoid harm. The cleaning station might be considered a useful resource by mantas as individuals regularly control access to cleaning stations through implied and overt aggression (behaviours used by other species to control territory (Wittenberger 1981)) via the agonistic displays observed between mantas which are described in more detail in Chapter 9.

Learning behaviour is well established in other elasmobranchs (Guttridge et al. 2009b). A variety of shark species have been shown capable of learning associations as rapidly as other vertebrates and remember training regimes for several months. Additionally, ecotourism and fisheries activities may lead to conditioning of sharks. However "social learning", or learning through the observation of another animal, whilst a well-documented phenomenon in the animal kingdom (Heyes and Galef 1996), has only been anecdotally observed in elasmobranchs and remains empirically untested. In the Maldives, "manta-watching" has been a popular tourist activity for nearly 40 years and some mantas have become accustomed to scuba divers and will continue to be cleaned when divers are present. Habituation also appears to have initiated some new behaviour. Exhaled bubbles by scuba divers regularly appear to be used by some mantas as a cleaning aid and some mantas will actively swim through, or hover in, exhaled bubble

streams; apparently to dislodge skin parasites from the gill surfaces. The bubbles can look like small jellyfish which is a known food of mantas (pers. obs.) and it is possible that this (presumed-cleaning) activity originated from mantas attempting to eat bubbles. Whale sharks *Rhincodon typus* are also sometimes observed attempting to feed on diver's exhaled bubbles and are known to be omnivorous (Silas and Rajagopalan 1963). A few mantas ingest bubbles and force them through the gill-slits which may help dislodge parasites on the internal surfaces. These behaviours are observed very frequently (>90% surveys) at most sites. There have been a few occasions when a smaller manta has been observed following a larger manta through a bubble stream, with the larger manta apparently teaching skills to the smaller one. This behaviour is indicative of social learning in this species. It is likely that the skills are also independently learned. In view of the commonness that the bubbling behaviour is observed in mantas, further research is warranted to quantify the number of animals performing the behaviour. Observations were not routinely recorded in this study, so the specific data on which animals perform this behaviour, and which ones do not, is not available. The data should be investigated to look for relationships between the mantas, there may be evidence which might support the social learning hypothesis, or a lack of it, with regards to this behaviour.

7.8 Next steps

A great deal of information on cleaning ecology by *Manta alfredi* has been gleaned from this study. Due to a lack of published data on elasmobranchs cleaning activity as clients, there is little with which to compare. Most reports of cleaning behaviour focus on the cleaners rather than the clients. Nevertheless, some important aspects of manta cleaning ecology are now recorded and some of the initial findings highlight gaps in knowledge and also provide a basis for future research. There are a number of shortcomings in this study, principally due a lack of knowledge on which to base initial hypotheses for testing. Many of the results from this study have not been as expected (effect of tide, size of cleaning stations relationship) whilst others (e.g. the effects of current) are so imprinted that surveys were not performed during strong current conditions so there is lack of data to support the relationship and thus hypotheses on non-uniform distribution of cleaning activity related to time of day and state of tide could not be properly tested.

New observations should be made by conducting full day surveys on (minimum) 3 days when the tidal range is greatest so that the effect of current may be investigated when high tide occurs mid-afternoon (approx. 15:00 hours) and another 3 days when it occurs mid-morning (approx. 09:00 hours) at Lankan. The surveys should be carried out in as similar conditions as possible i.e. weather, time of year, similar tidal amplitude. This will provide a useful dataset for Lankan. The studies should also be carried out at two or three other sites to look for general trends. They should be designed to have scientifically valuable results for individual sites in case general trends are not found.

A new study to measure the actual time being cleaned by mantas during attendance at a cleaning station, i.e. the time when the mantas had cleaner fish on, or around it (as opposed to just being in the vicinity of the cleaning station) should be undertaken to ascertain the actual time spent being cleaned. The study could also identify and investigate the other activities and behaviours of mantas whilst in the vicinity of the cleaning station (when not being-cleaned) in order to determine the proportion of time actually spent being-cleaned and the proportion of time performing each of other activities (swimming around but not being cleaned, social interactions etc.) as well. These may provide valuable insights into manta social behaviour.

Chapter 8: Preliminary observations of feeding behaviour of Alfred mantas (*Manta alfredi*) in the Maldives

8.1 Abstract

Snorkelling and scuba diving with feeding mantas is a common practice in the Maldives yet very little is known about manta feeding behaviour. Mantas are known to feed during both the day and night, so three study sites were selected to represent the range of environments where mantas have been known to feed. Mantas adopt a specific posture when feeding with the cephalic fins held widely open in the shape of an "O" and the mouth wide open. The posture is differentiated from the being-cleaned posture by the fin-tips meeting or slightly over-lapping; the mouth held widely open; an absence of cleaner-fish in the vicinity of the manta; and the swim speed of c. 30 bpm, being nearly twice as fast as when being-cleaned.

It is likely that feeding is commonly a group activity performed by small to large schools of mantas. Several types of movement were observed. Feeding whilst moving in a horizontal plane was most commonly reported, and usually performed by trains of mantas (a number of mantas following a lead animal in a line) passing through concentrated areas of plankton. Somersaulting on the same axis, or more rarely barrel-rolling (somersaulting in a horizontal or vertical direction), whilst feeding were also reported. Vertical ascents and descents whilst feeding were observed when the plankton layer was relatively deep. During an event with 100-150 mantas feeding together, simultaneous horizontal movements were observed. The mantas appeared to behave as a school, moving their wings synchronously. It is likely that all these movements are foraging strategies, the different movements being utilised to maximise feeding opportunity depending on location of food source.

8.2 Introduction

There is little published information about the feeding behaviour of either *Manta* species despite the large number of tourist interactions which occur with feeding mantas, both in the Maldives and worldwide. The knowledge gleaned could be incorporated into manta tourism policies and practices to maximise observations and develop tourism whilst minimising impact on wildlife. Best-practices for scuba diving and snorkelling with mantas and management of Marine Protected Areas (MPAs) including manta feeding areas, needs to be based on protecting the mantas and

preserving their environment but little is currently known about their requirements or behaviours and how human presence may impact these.

The species were not separated until 2009 (Marshall et al. 2009), thus all published reports mentioning feeding, may refer to one, or a combination of species (Bertolini 1933; Bigelow and Schroeder 1953; Compagno 1997). Mantas, in common with the other large planktivores, are ram feeders; swimming forward with their mouth open, engulfing the prey along with the water surrounding it (Sanderson and Wassersug 1993; Cortés et al. 2008). Telemetry studies by Rubin et al. (2008) of *M. birostris* indicated nocturnal feeding in deep water (50-450 m), so the activity is not easily observed by recreational scuba divers as the maximum recreational dive depth recommended by most agencies is 40 m. *M. alfredi* were reported by Clark (2008) feeding offshore at night but depth telemetry studies were not conducted thus the depths attained were unknown. Travelling offshore to deeper water to feed at night by *M. alfredi* was also implied by Yano et al. (2000) and Dewar et al. (2008) who suggested it was part of a daily migration, with mantas travelling to deeper water at night to feed and visiting shallow inshore waters during the day to be-cleaned and to feed. There is one verbal report by the divemaster on a private yacht in the Maldives which deployed a submersible remotely-operated-vehicle (ROV) at night, and observed around 20 *M. alfredi* feeding on euphausiid crustaceans (krill) at a depth of 200 m near Dhangethi Island in Ari atoll (Shaff Naeem, pers. comm.). Thus, it appears that mantas of both species may be primarily nocturnal feeders, travelling offshore to feed in relatively deep water. It is known that zooplankton migrates upwards at night (Cushing 1951; Forward 1988) so travelling offshore for night feeding is logical as the zooplankton would be more accessible at night.

Night feeding by mantas is a recently reported phenomenon and difficulty in monitoring mantas at the depths suggested and manoeuvring boats to research areas at night may limit future research on this subject. Plankton is attracted to lights, and underwater or surface lights may be placed to attract mantas to feed on the plankton in shallow (<20 m) depth. This has been a recreational scuba diving attraction in Hawaii for over 10 years (Neil 2007), where this learned behaviour by mantas was first described after a brightly lit hotel sign was erected next to the sea near Kona. Mantas were soon observed feeding in shallow water underneath the sign at night. The local dive operators then attracted the mantas to approach more closely by placing additional lights underwater

on the sea bed (7 m depth) underneath the sign, thereby guaranteeing a manta encounter for paying clients. The lights attracted swarms of small copepods on which the mantas fed. This activity continues to be observed nightly using the underwater lights as the hotel sign has since gone. In the Maldives there are many sites where mantas are known to feed on plankton attracted by lights placed for navigation purposes including underneath resort jetties over lagoons, behind boats at anchorage in lagoons and at ferry terminals e.g. Hulhumale and Hulhule Airport main jetties. Manta feeding activity at these sites typically starts at least two hours after sunset and continues until at least 02:00 h.

Mantas also feed in shallow water during the daytime (Ishihara and Homma 1995; Clark 2008; Dewar et al. 2008). Daytime feeding was frequently observed in the Maldives in areas on the leeside of atolls where localised areas of high plankton concentration may be caused by plankton being sucked into the atoll during rising tides, and reef structures restricting outflow as described in Chapter 3 and by Anderson et al. (2011). Under the conditions which may occur in March and October in the Maldives, large aggregations of several hundred mantas (a massing) were attracted to feed on the unusually large amount of zooplankton occurring in depths above 30 m and thus observed by recreational scuba divers. Whilst the purpose of aggregation is uncertain, aggregations of plankton feeders have been reported forming to take advantage of plankton abundance, and using the opportunity of massed numbers for reproductive purposes. The species reported were whale sharks *Rhincodon typus* (Nelson and Eckert 2007) and basking sharks *Cetorhinus maximus* (Sims et al. 2000). A massing event involving mantas is discussed here specifically for the feeding behaviours involved.

Ari and Correia (2008) described the surface feeding of captive *M. birostris* with open cephalic fins; however the manta was forced to remain on the surface when feeding as that was where the food was placed. The position of the fins was interpreted as motivation to feed. Interpreting cephalic fin position as a method of identifying behaviour was introduced in Chapter 7 and the hypothesis that there is a specific feeding position is investigated in this study. Duffy and Abbott (2003) included a photograph of a manta (*M. birostris*) with the caption describing it as “somersault feeding” just under the surface. The behaviour was not discussed further. Manta somersaulting was also briefly mentioned by Bigelow and Schroeder (1953) and Michael (1993b). I hypothesise that special movements when feeding are foraging

strategies and several types of movement observed are reported and discussed. Mantas appear to swim faster when feeding than cleaning and this hypothesis is also investigated.

Mantas are mainly plankton feeders but have been described as also feeding on small and moderate sized fishes ("small mullets") (Bertolini 1933; Bigelow and Schroeder 1953; Compagno 1997) although the size range of moderate-sized fish was not defined. There does not appear to be a specific study on the food of mantas thus their natural diet is not well understood but will not be investigated in this study except to report the plankton encountered. Captive mantas (likely *M. alfredi* from evaluation of photographs of individuals at Okinawa aquarium) are known to consume 12.7% of their body weight in euphausiids each week (Homma et al. 1999).

This Chapter describes three feeding events which include feeding behaviours commonly encountered in the Maldives:
1) Manta night-feeding activity caused by the placement of lights over water at Olhuveli resort, attracting plankton to surface water; described here in order to document some night time feeding behaviour by *M. alfredi* even though the environment is man-made.
2) A daytime feeding observation at Guraidhoo Kandu in South Male atoll, to describe typical shallow water feeding behaviour by mantas. This type of feeding behaviour is the most commonly observed in the Maldives, occurring daily at a number of sites distributed throughout most atolls and supports a significant snorkelling-with-mantas industry (Anderson et al. 2010).
3) An aggregation or massing event at Koshibee Kanduoli. This type of activity is less commonly observed than the typical daytime feeding (2), being reported by marine biologists and divemasters as occurring only several times each year. Similar events may be observed several times each month in Hanifaru lagoon, however the depth of lagoon (25 m) restricts some of the movements described here so the former example was used in preference. It is important because it is likely to be representative of night feeding activity in deeper water.

8.3 Method

All three survey sites are described as none are amongst the cleaning stations described in Chapter 3. At Guraidhoo the events were documented whilst skin diving using a housed Canon D300 digital stills camera and a Sony digital video camera. In the other

two examples the events were recorded by observers on scuba using a Sony video camera to record the event and notes were made on an underwater slate. Stills were taken from the video footage.

8.3.1 *Daytime feeding*

The feeding event was observed in Guraidhoo Kandu, South Male atoll (03º 53.960'N, 073º 27.758'E) on 19.10.2004. Similar events occur almost daily at this site throughout most of the SW monsoon (May to November). There is a nearby cleaning station further west inside the channel, but mantas were frequently observed feeding in shallow water anywhere from the point indicated in Figure 8.2 as "harbour entrance" to the western extreme of the site drawing. The feeding area encompasses a complex reef structure south of a natural harbour to the west of the Guraidhoo/Kandooma islands.

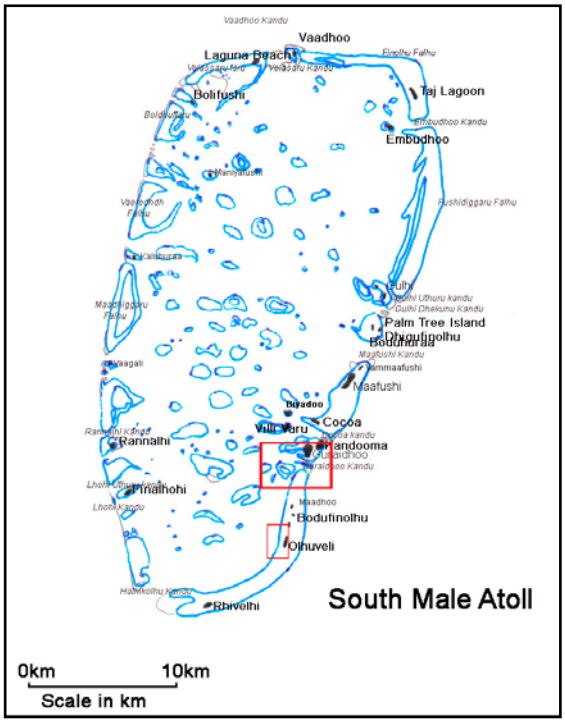


Figure 8.1 Map of South Male atoll. Upper red box indicates the vicinity of Guraidhoo Kandu and small, lower red box indicates the vicinity of Olhuveli island.

The survey was carried out whilst snorkelling to observe the mantas feeding (a common tourist activity at this location). Current strength was estimated using a hand-held GPS on the diving support vessel and speed in knots converted to $m.s^{-1}$. Manta swimming speeds were determined using the timer on the video sequence and counting the number of wing beats during each video sequence and calculating beats-per-minute. A beat was defined as one complete cycle of pectoral fin movement, moving from horizontal to its lowermost deflection through to its uppermost deflection and back to horizontal. Stills and video footage were analysed for specific behaviours (see results section 8.4.1).

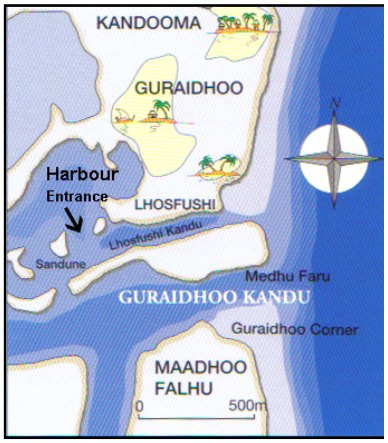


Figure 8.2 Diagram of Guraidhoo Kandu from Dive Maldives (Godfrey 2006). The north section of the channel is known as Lhosfushi Kandu and includes "Harbour entrance" where mantas were observed during the incident reported. Mantas may be found feeding anywhere within the channel or within the harbour area. The harbour is a lagoon in a faro-like structure.

8.3.2 *Night feeding observation*

The night time feeding event was at Olhuveli reef 3º 51.000'N, 73º 27.144'E on 25.3.2008. The dive site is known as 'Three Moons/ Tineh Handu (in *Dhivehi*)' due to the presence of the three jetty lights which appear as artificial moons when under water. The bright lights concentrate plankton into the shallow water (0-5 m depth) immediately below the lights. Mantas may be sighted throughout the year at this site, when surface conditions are calm. The jetty slightly overhangs the fringing reef of the western lagoon surrounding the islands of Bodufinolhu, Olhuveli and Rhivelhi. The west-facing lagoon fringing reef wall falls almost vertically to a depth of around 12 m and then continues more gradually to a depth of around 30 m inside the atoll. To the east of the fringing reef is a 2-5 m deep lagoon. Diving was conducted from a boat positioned 50 m south of the jetty adjacent to the inner fringing reef outer crest and entry to the water made so as not to disturb mantas feeding. Diving commenced after 21:30 as mantas are not usually observed before 20:30 (sunset was at 18:23). Observations were carried out at depths of 3-10 m, immediately below the jetty; without the use of dive lights which may cause disorientation of mantas. The ambient light produced by the jetty lights was sufficient to video, observe and identify individual mantas from their ventral markings patterns, using the method described in Chapter 4. To cause minimal disturbance to the mantas the observation period was limited to 30 minutes. Video footage was analysed for specific behaviours and discussed in results section 8.4.2.

8.3.3 *Mass feeding event (massing)*

On 13.3.2008 from 15.15 to 16.45, a massing event was observed in the central area of Koshibee Kanduoli (*kanduoli* is the *Dhivehi* word for wide channel) 6º 18.430'N, 72º 58.912'E. The event was initially observed from the surface, then by snorkelling, and

then using scuba equipment. The event was captured on digital video using a Sony camera and the footage reviewed to analyse behaviours and identify individual mantas from ventral markings patterns. Dive computers were used to record depths of observations.

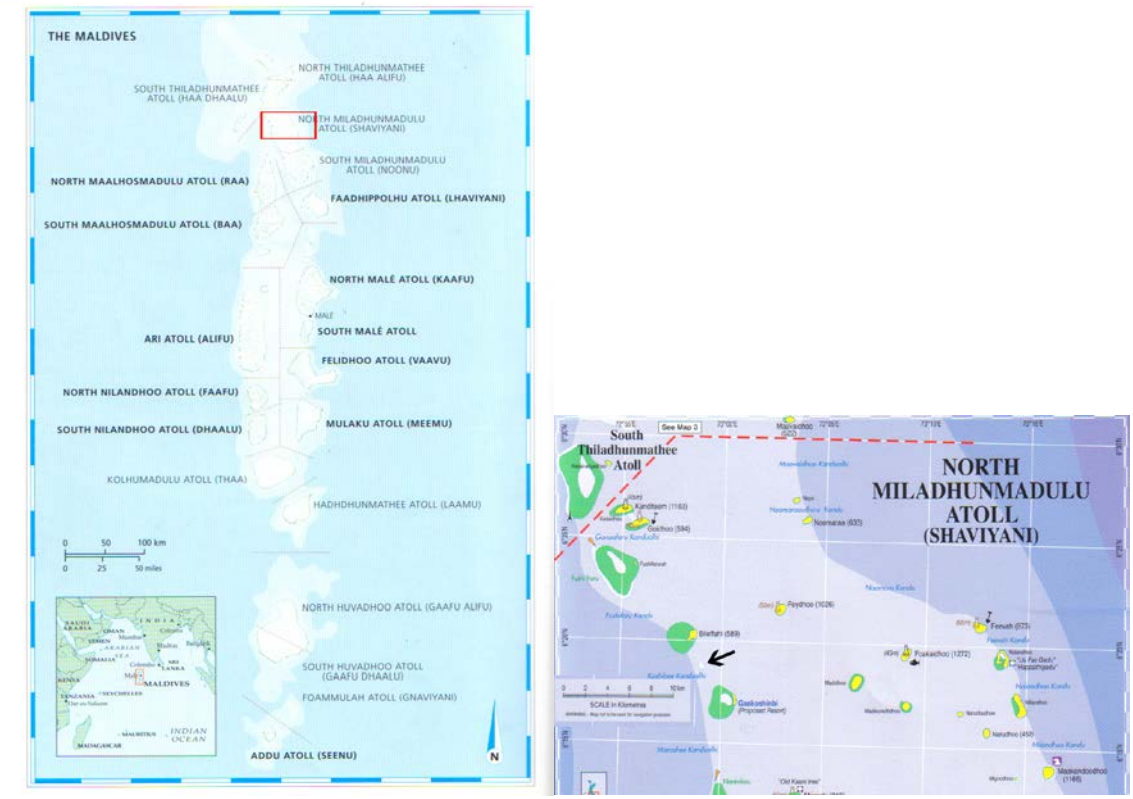


Figure 8.3 Map of Maldives (left) with Shaviyani atoll highlighted in red box (from Harwood and Bryning (1998)). Right is detail from area highlighted for Shaviyani atoll (from Godfrey (2004)). Black arrow indicates area in Koshibee Kanduoli (wide channel) where mantas were observed.

8.4 Results

8.4.1 *Daytime feeding at Guraidhoo Kandu*

10 mantas were observed feeding within the channel and the entrance to the natural harbour of Guraidhoo island. Feeding activity was identified by the mantas maintaining the cephalic fins in an open “O” position and the mouth held widely open (see Figures 8.4 and 8.6). The sizes of the mantas were between 2 and 3 m DW estimated by comparison with snorkelers next to them. During the survey, water was flowing out to the ocean (west to east) creating a back eddy over the section of the reef where the mantas were observed feeding. The current velocity was estimated as 1.32 $m.s^{-1}$ within the channel (strong), and estimated as 0.5 $m.s^{-1}$ in the eddy. Wave conditions were calm

as the area is sheltered. Water temperature was 28ºC. The depth of the water where mantas were observed varied from (estimated) 3 to 20 m around the slope of the shallow reef of the east harbour entrance. The water was not analysed to determine plankton type but contained gelatinous particles 0.5-1 cm in length and 0.1-0.2 cm in diameter. Twelve video sequences (5-22 s in length) were analysed to determine swim speeds. Mean swim speed was 29.25 beats per minute (bpm) (SD ± 4.82, median 30.0).

Figure 8.4 Photograph of manta feeding below surface. Mantas were observed feeding within 1 m of surface for (estimated) >95% of time during survey at Guraidhoo Kandu. Mouths were maintained in fully open position as shown.

Occasionally, feeding was observed at 5-7 m depth but the vast majority (estimated >95% time observed) of feeding was within 1 m of the surface, as in Figure 8.4. A feeding strategy, which appears to be a variation of echelon feeding (Wursig et al. 1986; Wuersig et al. 2002 ; Moore et al. 2010), was observed. The group of mantas were observed following a lead manta in a line, swimming through areas of high turbidity (assumed to contain high concentrations of plankton) whilst actively feeding (assessed by mouth and cephalic fins held open). After making a sweep through the plankton, the group then turned around and made a return sweep through the area of plankton. I have named this movement **horizontal feeding** (feeding in a horizontal plane) in a **feeding chain** (a line of mantas in a feeding scenario- the term train has been used to describe lines of males chasing a female during courtship (Yano et al. 1999b) so a different word is used relation to feeding behaviour). Echelons are a diagonal or V-shaped formation commonly observed by pods of cetaceans moving in shallow water (or birds in flight). The chain of mantas passed to and fro, making parallel forays through the plankton concentration (Figure 8.5).

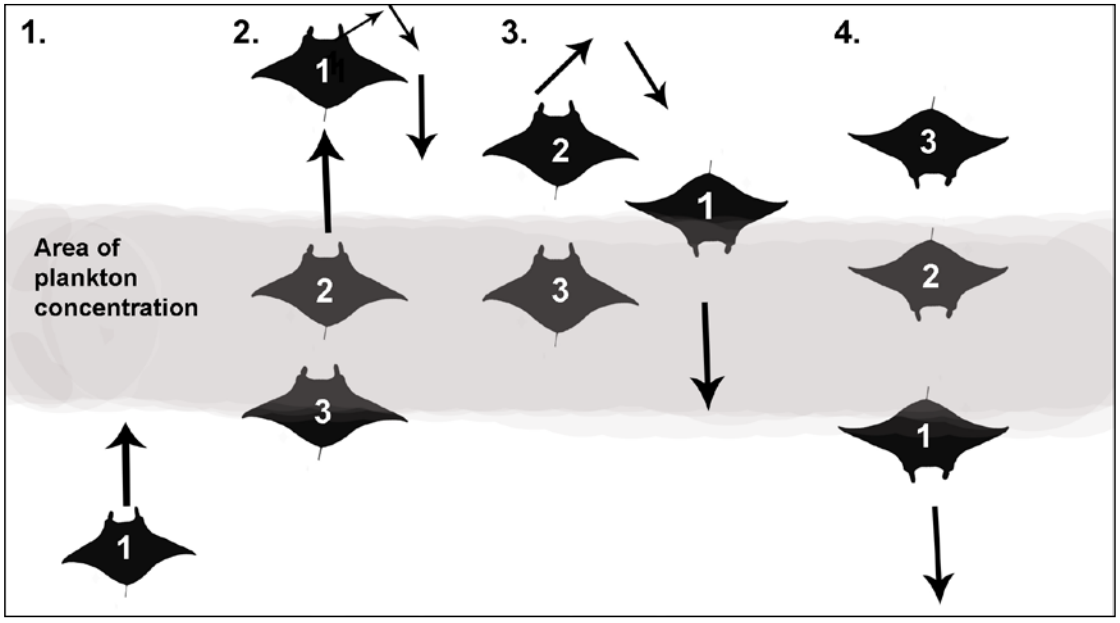


Figure 8.5 Four steps in a sequence representing the swim pattern by mantas observed at Guraidhoo Kandu. (1.) Manta 1 approached area of high plankton and swam through whilst feeding, (2.) followed by mantas 2 and 3. (3.) Manta 1 then turned to swim back through the area of high plankton concentration, (4.) followed by mantas 2 and 3. I have termed this behaviour "horizontal feeding".

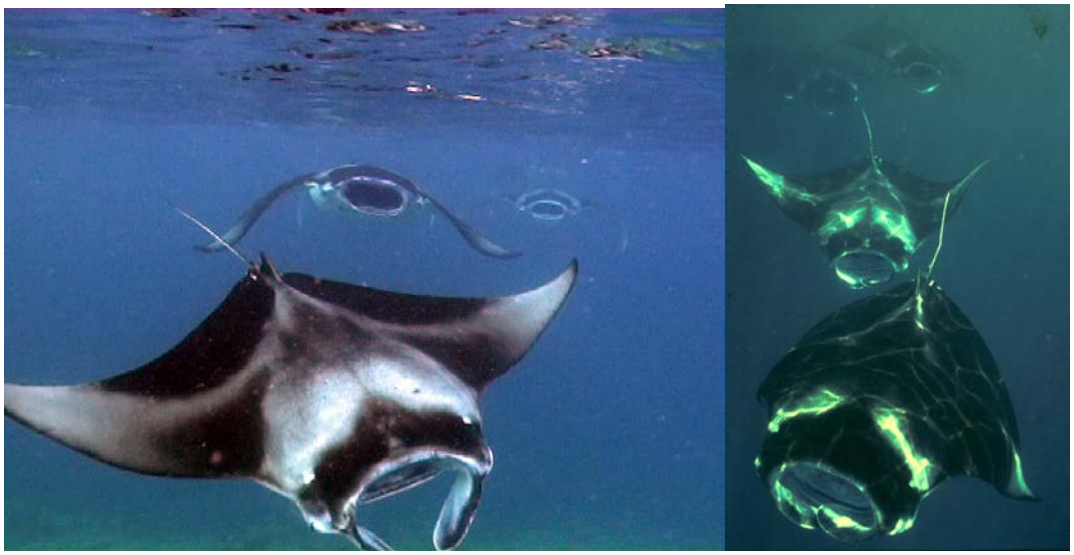


Figure 8.6 Photographs of horizontal feeding by mantas. Mantas form a line (chain), following lead manta making horizontal line swims through plankton. Although chains are slightly staggered, following mantas remain in similar a plane to preceding mantas.

The line was slightly staggered on some occasions, with following mantas slightly (<< width/height of a manta) left or right, shallower or deeper than the previous manta (Figure 8.6). Thus, the chain of mantas appeared to swim through approximately the same plane as the animals previous to them. A chain of up to 7 mantas was seen to form

at any one time. All maintained their mouths and cephalic fins fully open (open-feeding position, section 7.6.8) for the majority of the observation period (estimate >95% time observed). With the mouth wide open, the gill-bars were clearly visible inside the buccal cavity (Figure 8.7 left). The cephalic fin position when feeding differs to the relaxed, open position adopted when a manta is being cleaned as the tips of the cephalic fins usually touched and often overlapped (Figure 8.7).

Figure 8.7 Left manta has cephalic fins in exaggerated 'O' open-feeding position. The mouth is wide open and the gill-bars can be seen in the buccal cavity. Right: in contrast, the manta has cephalic fins loosely held, in the relaxed open-cleaning position: the mouth is barely open.

It was frequently noted that feeding mantas would approach snorkelers. If the snorkelers remained passively on the surface (i.e. they did not swim after the mantas), different mantas would swim over to the snorkeler and then dive under the snorkeler (Figure 8.8).

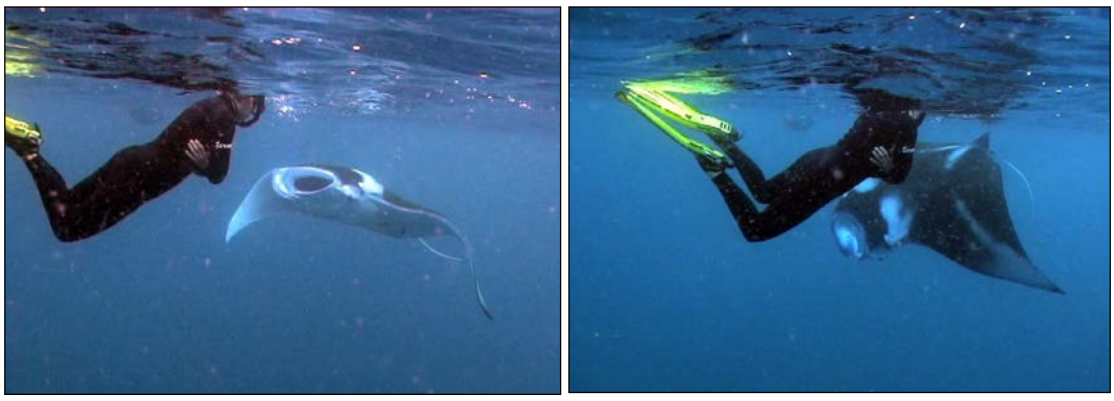


Figure 8.8 Left: manta approaches a stationary snorkeler waiting on the surface. Right: manta swims underneath the snorkeler.

8.4.2 *Night time feeding at Olhuveli*

Ten mantas were observed feeding at the house reef of Olhuveli resort on 25.3.2008. On the night of the observation, winds were 8-10 knots (4-5 $m.s^{-1}$) SW, water temperature

28ºC, sea condition calm to moderate. The observation period commenced at 21:30 h. The mantas varied in size from 2.0 to 3.25 m DW (estimated by comparison with divers nearby) and males and females were present although only five females were positively identified. Twenty-five sequences of feeding mantas were analysed. The mean swim speed was 28.95 bpm (SD ± 5.93, median 29.2).

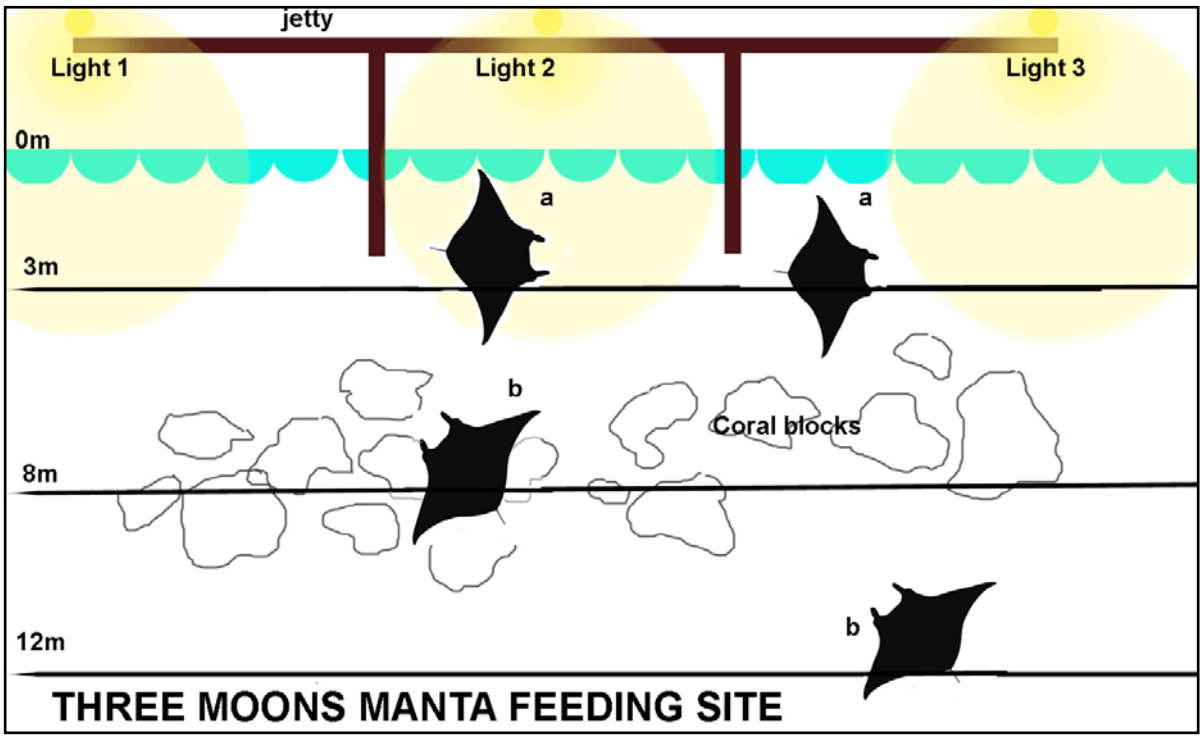


Figure 8.9 Diagram of Three Moons reef to show two types of swimming behaviour: a and b. Mantas (a) were feeding in very shallow water (0.5 to 3 m depth) making horizontal sweeps (as in horizontal feeding), often in feeding chains of two or three animals directly under the lights. Mantas (b) made semi-vertical swim patterns, very close to the reef contour and appeared to be hunting close to the coral blocks.

The video was reviewed for interesting behaviours. Two main swim patterns were observed (Figure 8.9). Mantas either made horizontal swims (a), directly under the lights, whilst remaining very shallow (surface to 3 m depth), or made diagonal, semi-vertical dives (b), apparently using the reef slope as a guide and typically remaining 0.5-1 m from the reef, hugging the reef contour. I have termed this type of movement **hunting-feeding**. The semi-vertical dives were made from 3 to12 m depth and were also made in reverse, from depth towards the surface. The swimming close to the reef and past divers appeared very deliberate and similar to hunting activity observed in reef sharks or stingrays when feeding near the bottom. This type of foraging behaviour has not been previously described in mantas. The corals did not appear to be spawning, but

there were a large number of small baitfish (likely juvenile mackerels (Scombridae)) present, which were being hunted by jacks, as well by the mantas. The mantas may also have been attempting to feed on small fish hiding close to the reef.

Horizontal feeding by a train of mantas in pairs or threes were observed several times and along with solo horizontal feeding, was the most common activity observed (estimate 70% time). Some somersaulting was observed, particularly in very shallow water. Most of the somersaults were used for manoeuvring rather than feeding (i.e. a half-somersault to change direction, followed by horizontal feeding near the surface).

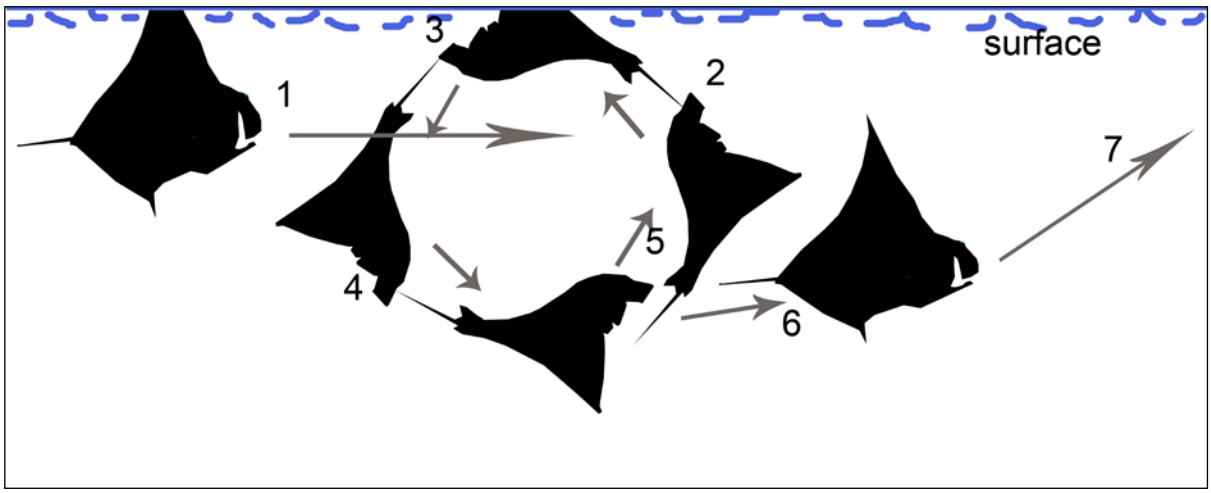


Figure 8.10 A surface somersaulting sequence. Manta (1) swims forward then swims upward flexing backwards (2) to apex position where dorsal wings or abdomen may break surface (3). The backward somersault continues (4) through to bottom apex and then the manta swims upwards again (5) and through stages (2) to (5) again. The somersault may be repeated several times before forward motion (6) to (7) occurs.

When surface somersaulting and feeding (i.e. not for manoeuvring) the rotation was made in the same place several times i.e. somersaulting several times on the same axis. A somersault movement is sometimes termed a barrel-roll, but the latter implies forward movement as well as a 360 degree roll. The somersaults when feeding were stereotypical in that the same axis was maintained, either at a point just under the surface, but typically 1-5 m depth. This same-axis roll whilst feeding is termed **somersault feeding** (a third feeding strategy) following Bigelow and Schroeder (1953) and Duffy and Abbott (2003) and described in Figure 8.10. The manta swam at a normal feeding swim-speed whilst somersaulting, so the performance appeared dynamic. This feeding strategy would be efficient for feeding on a small area of

concentrated plankton just under the surface. The mantas feeding just under the surface were feeding on a mixture of crab and shrimp larvae, and other unidentified zooplankton.

Four instances of mantas approaching scuba divers and swimming through bubble streams with mouths open were observed which appeared to be feeding rather than cleaning activity due to swim-speed and that the mantas held their mouths open. The mantas made several horizontal close passes (within 2 m) of divers whilst continuing to feed.

8.4.3 *Mass feeding event in Koshibee Kanduoli, Shaviyani Atoll*

Observations commenced at 15:00 h with the sighting of 20-30 mantas horizontal feeding on the surface in long trains. The feeding was observed for approximately 20 minutes before an entry into the water was made. With no reef reference, the strength of current could not be determined. On the previous day, a very strong current (estimated > 1.32 m sec^{-1}) was observed at the same time of day on the east side of Raa atoll, located approximately 60 km south west from this location. This is noted because it is known that strong currents may create convergences and upwelling of nutrients (Anderson et al. 2011). The mantas were of estimated size 2.5 to 4.5 m DW. There appeared to be more large adults present by proportion than at Guraidhoo or Three Moons. Of 23 individuals whose sex could be determined, 20 were female and 3 were male.

Once in the water (snorkelling) it was clear that the 20-30 mantas observed from the surface were part of a large group of mantas (~100) mass feeding, and the majority of mantas were below the surface at depths of 5 to 20+ m. We observed this large group for ~25 minutes. The plankton was so thick that is was very difficult to see below 20 m depth (Figures 8.11 and 8.12) so a skin dive was made to check conditions and it was noted that the plankton was significantly thicker below 10 m depth, causing reduced horizontal and vertical visibility (<15 m). The mantas made vertical dives (which I have termed **vertical feeding**) from the more transparent water above 10 m into the thicker plankton layer and then made somersaults, both in the same axis and barrel-rolling, in and out of the plankton: the feeding movement was vertical as well as horizontal. Four mantas made a direct vertical ascent travelling 15 m whilst feeding, one after the other, as if in sequence (Figure 8.11). Despite observations of vertical movement, most swimming was in a horizontal plane (est. >95%).

Simultaneous horizontal feeding by many mantas was observed at all visible depths so that some stacking of mantas appeared to occur due to the great density of animals present. This appears to be a variation of the horizontal feeding strategy. Multiple chains at different depths created stacks. In some cases the stacking seemed deliberate, with males and females appearing to ride on the back of other animals in behaviour very similar to that of male shadowing of females during courtship (see section 10.3.3). Stacks of up to four animals occurred. Feeding behaviour continued throughout the deliberate stacking so it was deduced that the priority was feeding and not mating. The number and density of animals was such that the group of mantas appeared like a tornado, spinning around some central point with a diameter of around 50 m and of similar height. Mantas were observed joining the tornado by swimming in perpendicular to the movement of the main body and then turning through 90 degrees to join the main group. After 25 minutes the group of mantas dispersed but had remained in a tight formation throughout the period of observation.

After around 10 minutes, another group of mantas was observed approximately 200 m distance from where the previous group had dispersed, and an entry to observe them using scuba equipment was made. This was the largest group of mantas observed so far, with an estimate of 120-150 animals mass feeding together. The group of mantas occupied depths from the surface to approximately 50 m depth (visual estimate). The plankton appeared evenly distributed below 10 m although reduced penetration of light below 30 m made plankton abundance estimation more difficult. The feeding behaviour changed during the observation period of ~ 30 minutes. During the early period, smaller groups of mantas (up to ~30 mantas) made foraging parties through the plankton with less obvious patterns of movement. During the latter part, the majority of mantas aggregated into a tornado formation again and this was maintained throughout the latter part of the in-water observation. Smaller groups of mantas (twos and threes) were observed breaking away from the main tornado and making somersaults (around the same axis) at depths of 10-20 m. Vertical feeding descents were observed involving a manta commencing a somersault manoeuvre but continuing downwards after the turn was completed (Figure 8.14). In one sequence a manta started a backwards roll making a vertical descent into the plankton, and was followed by 7 other mantas in loose sequence. In a second sequence 17 mantas were observed making backward vertical descents into the plankton. This behaviour was very distinctive due to the white flash of

the ventral surface as the mantas backward rolled (Figure 8.13). Vertical feeding descents were difficult to observe in their entirety due to poor visibility (Figure 8.16).

Figure 8.11 Manta feeding whilst making vertical ascent.

Figure 8.12 View downwards from surface. A high density of mantas is apparent but plankton and poor light penetration makes observation beyond 20 m depth difficult.

In addition to stacking within the tornado, nearby mantas swam with synchronised wing motion and swim speed to avoid collision whilst maintaining feeding momentum. Figure 8.15 shows approximately five mantas in tight formation with their wings in the up movement of the beat cycle. The proximity of the mantas and the synchronous swimming would indicate that the mantas formed a school based on the definition of Partridge (1982) whereby a school is a group of three or more fish in which each member constantly adjusts its speed and direction according to the other members of the school. Other definitions of school require groups of fish to be characterised by polarised, equally spaced individuals, swimming synchronously with inter-individual distance < 1 body length (Breder 1976; Pitcher and Partridge 1979; Pitcher 1983) which describes the behaviour of mantas in these large feeding aggregations when swimming in the tornado formation.

A plankton sample was not taken for analysis however there were no obvious crustacean (including shrimp or crab larvae), juvenile fish or Cnidaria present. Swim speeds at Koshibee averaged 29.7 bpm (SD ± 5.72, median 30.2, n = 22).

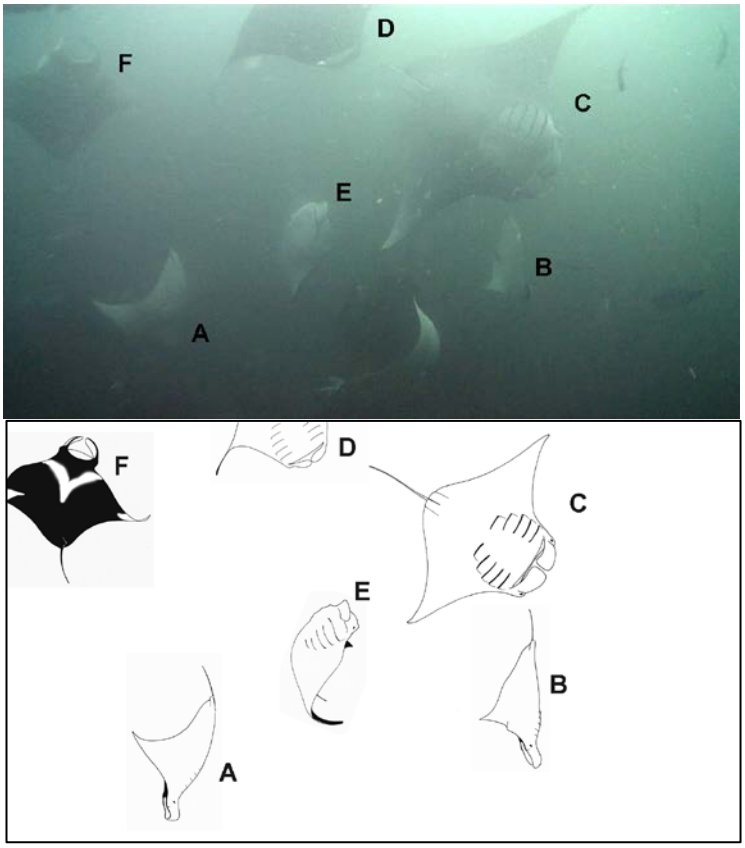


Figure 8.13 Photograph and illustration (for clarity) of a sequence showing flashing of white ventral surfaces during somersault feeding followed by a vertical descent. Animals are lettered in sequence. Mantas A and B have already somersaulted backwards and are descending whilst feeding. Mantas C and D have just turned over and are starting to descend whilst mantas E and F are commencing their backward somersaults. Illustration by Jacquie Briggs-Morris.

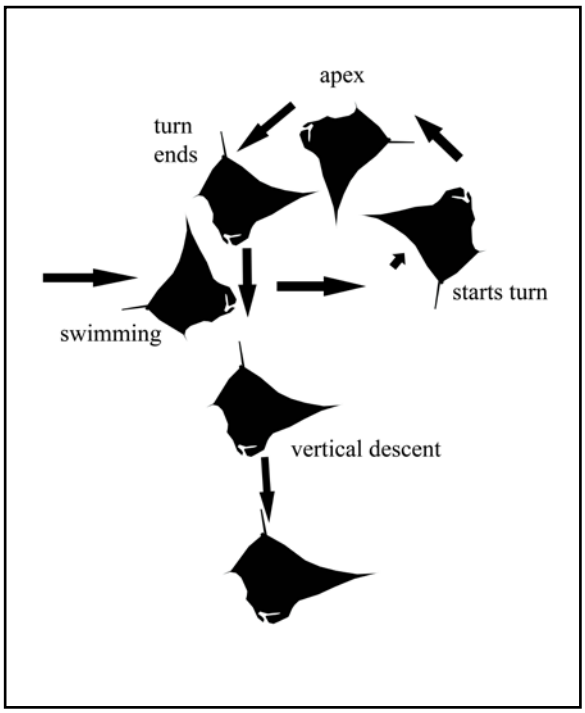


Figure 8.14 Diagram of vertical feeding descent. Manta starts turn, reaches apex and turns over is if somersault feeding (Figure 8.10) but stops turn and continues downwards in a vertical movement continuing for a further 5-20 m distance.

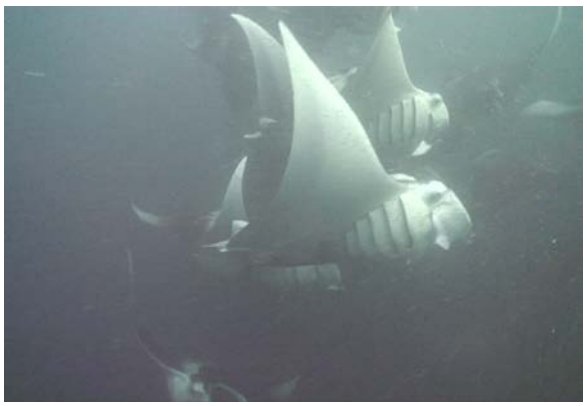


Figure 8.15 Synchronised swimming was observed within the tornado. Here, at least five mantas are swimming in tight formation and their wings are all in the up position of the beat cycle to avoid collision and maintain feeding momentum.

Figure 8.16 Upwards view from 20 m depth. At least 25 mantas can be counted in this frame. Large unidentified plankton particles are obvious

8.4.4 *Stereotypical feeding swim speed*

Swimming speed whilst feeding at each of the three sites were compared using one-way ANOVA after confirming normal distribution of results. The swim speeds at the three sites were not significantly different (F = 0.17, P= 0.844) and swim speed data for the three sites were pooled and checked for normality using Anderson-Darling test: AD = 0.181, P = 0.91. Thus, the combined data is also normally distributed. The mean feeding swimming speed at the three sites was 29.18 bpm (SD ± 5.54, n = 58).

The mean feeding swim speed was compared to the mean swimming speed whilst being cleaned (section 7.3.6) (n = 58, mean 16.14 bpm, SD ± 3.46) by applying a *t*- test. The mean swim speed when feeding was significantly faster than whilst being cleaned: t = 15.22, P < 0.001, DF = 95. This result supports the observation that mantas appear to swim more purposefully (faster) when feeding.

8.5 Discussion

8.5.1 *Stereotypical feeding, cephalic fins posture and swimming speed*

The posture of the cephalic fins held widely open in the shape of an "O" with the mouth wide open, whilst feeding, is an iconic image of manta rays; introduced in Chapter 7 when compared with the relaxed/open pose adopted whilst being cleaned. To differentiate the feeding posture: (1) the cephalic fins were maintained in a wide "O" position with the tips meeting or slightly over-lapping; (2) the mouth was widely open (as shown in nearly all photographs in this Chapter and particularly in Figure 8.7); (3) there was an absence of cleaner-fish in the vicinity of the manta and (4) swim speeds (beats per minute bpm) when feeding were nearly twice as fast as when being-cleaned. This faster speed corroborates with a ram-feeding mechanism. The mantas drove themselves through the water, forcing water through their gills to strain out the plankton. Additionally, feeding was a dynamic activity involving significant horizontal and/or vertical motion whilst cleaning involves slower swimming over a confined area and much hovering. All observations of feeding involved fast, forward swimming.

Swimming speeds measured during the three reported feeding episodes were similar despite the different circumstances. The swim speed whilst being-cleaned was more likely to be influenced by local currents as the mantas needed to hover over a fixed area and current speeds varied from day to day from site to site. Plankton (their food source) moves in the same water mass as the mantas, so swim speed when feeding was less likely to be affected by variations in local currents. The open cephalic fin and mouth position, accompanied by a swimming speed c. 30 bpm may be interpreted as stereotypical feeding behaviour in *M. alfredi*.

8.5.2 *Feeding strategies*

Congregating is one of the most striking and familiar aspects of animal behaviour and commonly observed in fish (Bertram 1978; Pulliam and Caraco 1984; Hoare et al. 2000). Fish school shapes and dynamics may be related to the avoidance of predators, feeding, migration, energy conservation and reproductive behaviour (Blaxter and Hunter 1982). The line-style swim patterns of mantas when feeding had not been previously described, but appear to be schooling formations employed to optimise feeding on plankton in shallow water. To differentiate horizontal line feeding from diagonal or V-shaped echelons performed by cetaceans (Wursig et al. 1986; Moore et al. 2010) the line-formations have been termed chains to differentiate it from the

terminology used to describe the lines of males following females during courtship. Horizontal chain feeding by mantas is the most common form of feeding strategy. More complicated three dimensional aggregations and schools of mantas were formed when the food source was present in much deeper layers as was observed at Koshibee (e.g. stacking, tornado shaped formation, etc.). This more complicated aggregation appeared similar to typical fish schools. Fish schools are characterised by polarised, equally spaced individuals swimming synchronously (Breder 1976; Pitcher and Partridge 1979; Pitcher 1983). It appears that feeding is commonly a group activity performed by small-to-large schools of mantas; in contrast to cleaning which is commonly a solitary activity (Chapter 7).

Somersaulting movements by *M. birostris* when feeding was previously mentioned by Bigelow and Schroeder (1953) and Duffy and Abbott (2003) but not described, and somersaulting with a vertical descent or ascent had not been previously reported. It is likely that all these movements are foraging strategies with different movements being utilised to maximise feeding efficiency depending on the location of the food source. It has been shown that sharks may track biotic changes with some accuracy (Heithaus et al. 2007), with basking sharks actively selecting areas of high prey abundance over other areas (Sims et al. 2006). If the plankton was mainly located on or near the surface (e.g. caused by an upwelling over a reef) then surface feeding in horizontal trains would be an efficient feeding movement. Surface somersaulting (or somersaulting with a vertical descent) might be used to determine the depth of a plankton layer. Somersaulting could also be used to maximise feeding on a small volume of concentrated plankton. The stacked, tornado-shaped feeding observed at Koshibee would be useful when the plankton was located in a larger area, extending into deeper water. The vertical descents (and ascents) observed at Koshibee would be practical to identify the depth of the plankton layer. Mantas feeding in groups and following each other in close trains and stacks when feeding suggests co-operative feeding strategies. The feeding-train strategy initially appears inefficient as it would be expected that the first manta would deplete the food stock, however the fast swimming action and wing movement may mix water and actually direct plankton to the following manta's mouth. Plankton concentrations in a manta train warrant further investigation.

Sims and Quayle (1998) showed basking sharks *Cetorhinus maximus* choosing the richest, most profitable plankton patches, foraging along thermal fronts and actively

selecting areas that contain high densities of large zooplankton above a threshold density. Yopak and Frank (2009) related the small brains of the basking sharks and whale sharks *Rhincodon typus* to their opportunistic passive predation strategies, which might be less demanding cognitively in terms of sensory and/or motor requirements in comparison to more agile hunters. *M. birostris* has the largest brain weight and relative brain size amongst all chondrichthyans (Ari 2011) and the gross morphology of their brains resembles that of hammerhead sharks (*Sphyrna* sp.) "most likely due to the 3-dimensional habitat they live (in), their active and manoeuvrable lifestyles, highly developed social and migratory behaviour, and possibly the increased ability of sensory processing thanks to the broad shape of their heads". The size of the manta brain suggests the cognitive capability to hunt like hammerhead sharks, pursuing their food rather than passively swimming through areas of high food concentration in a manner similar to surface feeding by basking sharks or whale sharks. Elasmobranchs can detect natural electrical fields around their prey via the ampullae of Lorenzini (Kalmijn 1971; Fields 2007) which are known to exist on the dorsal and ventral disc of rays (Wueringer et al. 2009). Using this electro-sensory system they could orient to the field around prey in order to attack, even when visual, chemical and mechanical cues were absent. The avoidance of divers and reef during the night feeding showed very good visual acuity in low light levels by the mantas or the incorporation of their electro-sensory systems. The very close reef-hunting, with the mantas swimming within 0.5 m of the reef following the reef contour whilst continuing to feed was similar to hunting by sharks or stingrays. This is a behaviour not previously described in this genus.

The best known forms of visual communication in animals involve the display of distinctive body parts or distinctive bodily movements. Often these occur in combination, so a distinctive movement acts to reveal or emphasise a distinctive body part. Myrberg (1991) proposed that distinctive fin markings in many carcharhiniform sharks accentuates intraspecific social signals, and illustrated by the high visibility of the white fins tips of the oceanic white tip sharks *Carcharhinus longimanus* (Poey, 1861). The visual acuity of mantas has already been established by Ari and Correia (2008) and social learning (learning through observation of other animals) is a well-documented phenomenon throughout the animal kingdom (Heyes and Galef 1996) but has so far not been tested empirically in any elasmobranch (Guttridge et al. 2009b). That a single manta seemed to set off a chain of up to 17 mantas copying the initiator's behaviour is important. This behaviour may be a deliberate signal (or a direction) to

other mantas (the white flash) to imitate the lead animal. In mantas the ventral surface appears in bright contrast against the water and could provide a strong visual signal.

It appears that *M. alfredi* may incorporate the detection of chemical and biotic changes via swim patterns, use electro-sensory systems to detect prey when in close vicinity and use visual signals to communicate whilst feeding. Whilst these are preliminary conclusions which require further research, it does appear that manta feeding strategies are complex.

8.5.3 *Other observations from feeding activity by mantas*

Other planktivore elasmobranchs have been recorded taking advantage of conditions of food abundance for reproductive activity (Sims et al. 2000; Nelson and Eckert 2007). However, it was unknown whether the mating was incidental to the feeding, or these were locations where the majority of matings took place (i.e. a mating area). The mass feeding event reported here may have been used for pre-mating courtship activity as several stacks of mantas were observed. In the stacks, the mantas would appear to ride each other's dorsal surfaces whilst continuing to feed, in a manner similar to pre-copulatory shadowing (Chapter 10). Whilst the majority of activity was males on females, there were some examples of females on females and stacks of three and even four animals. As proposed in section 10.4.3, females may select males on their swimming speed, agility and endurance so these stacking events may be mantas practising their agility and co-ordination skills. No copulation activity was observed so it can be concluded that these aggregations were primarily for feeding.

The plankton being consumed at these locations varied and did not appear to contain zooplankton at the mass feeding event. Further investigation on the variety of natural food of mantas would be useful. These observations confirm *M. alfredi* consuming small shrimps and baitfish and they are likely to be omnivorous, as are whale sharks (Colman 1997). Whilst it is known that mantas (both *M. alfredi* and *M. birostris* species) travel to deeper water at night in order to feed (Yano et al. 2000; Clark 2008; Rubin et al. 2008), it is still unknown whether night is the main period of feeding, or whether they feed during both the day and night, when opportunities arise. The commonness of sightings of mantas feeding during the day, throughout the Maldives and elsewhere, suggests that feeding during the day is more than supplemental. Examination of the stomach contents of a number of mantas, in combination with improved knowledge on the variety of foods consumed via plankton trawls in areas

where mantas are observed feeding, might provide more information on their feeding ecology.

It appears that feeding events are not competitive, but may be co-operative events where mantas lead and follow each other to areas of best plankton and apply highly organised foraging and feeding methods to maximise food consumption. The feeding events may also be used for social interaction (mating skills practice) and swimming skill development. Future research to develop the theories on visual stimuli and co-operative behaviour should be conducted. An experiment to determine whether there is a positive response to white (i.e. a stronger or different response to white than to other colours) could be performed by monitoring the response of mantas in the same situation e.g. at a feeding or cleaning area to a large colour panel. Suggested colours would be white, black, yellow (as also highly reflective, RGB 255, 255, 0) and mid-grey (RGB 112, 112, 112). The response(s) with no panel would be the control. To investigate co-operative behaviour a time-spent-feeding experiment with particular attention to arrival and departure of individuals to see whether mantas arrived and departed as part of a group or as individuals (similar to the investigations in Chapter 9, made at a cleaning station). An investigation of the relationship of time spent feeding with number of other mantas present (similar to that conducted in 7.6.3) could be conducted. Food is a finite resource, once eaten it is gone. The quantity of food will therefore be a major factor controlling the time spent feeding in any study performed. Nevertheless, if a significant proportion of mantas feed on their own it would undermine the hypothesis that feeding is a co-operative, group behaviour.

Further research will be required before specific recommendations on tourism practices around feeding manta could be made as it appears that manta feeding behaviour is more complex than originally anticipated and new feeding strategies are being discovered. Feeding appears to be highly dynamic, often occurring in very shallow water so it would be important to manoeuvre boats carefully around feeding mantas and avoid placing snorkelers directly into groups of feeding mantas. Mantas are highly manoeuvrable. They should be able to avoid boat strikes and too-close contact with snorkelers and can move away if interactions become too invasive. Good practice by operators should involve passive observation from a respectful distance, so that any interaction is initiated by the wildlife.

Chapter 9. Preliminary investigations of social structure and observations of agonistic behaviours in Alfred mantas *(Manta alfredi)* in the Maldives

9.1 Abstract

The social structure of *Manta alfredi* in North Male atoll was investigated to identify whether there was any evidence of a social network and to identify whether there were trends in sociality with size and sex. The sightings of a sample of 84 individual mantas seen at least 5 times between November 2001 and November 2010 were analysed to determine an index of association (IA) between pairs of individuals. 4398 relationships were discovered (index of association (IA) > 0) but 2574 pairs had no relationship (IA = 0). IA between pairs of mantas ranged from 0 (never sighted together) to 0.417. The paired results for each animal were averaged (excluding zero results) to produce an individual mean IA. The average individual mean IA was 0.1004 (SD ± 0.0195) but the female average mean IA was significantly smaller than the male suggesting that males have stronger associations with other mantas. A positive correlation between mean IA and number of mantas associated with the individual and a negative correlation in mean IA and number of times seen suggests that there may be behaviour traits amongst mantas. “Bolder” mantas may be seen more frequently and have weaker associations with other mantas; and “shyer” mantas may be sighted less frequently, and when sighted, to be in the presence of other mantas. The arrival and departure of mantas to cleaning stations was investigated for evidence of group behaviour but results were inconclusive. The putative agonistic behaviour termed a “head-off” was described. This appears to be an agonistic display performed by two mantas in competition for access to a cleaning station. The mantas approached each other head-to-head and then reared up so as to be pose abdomen–to-abdomen and appeared to compare size. The larger manta (assessed by the extension of the wings) was victor, and the loser swam away or behaved in a submissive manner around the victor. Knowledge of this behaviour may be useful in managing recreational scuba divers in the presence of mantas.

9.2 Introduction

In the absence of any published literature on social structure or behaviour in either species of *Manta* or any other ray, a review of social behaviour of sharks (which appear to be the nearest elasmobranch relatives to have had their social behaviour studied) was conducted.

A number of individuals living and interacting together implies social behaviour (Krause and Ruxton 2002). There is evidence to suggest that sharks are capable of various forms of social recognition as sharks are known to group by size, sex and species (Klimley and Nelson 1981; Economakis and Lobel 1998; Sims 2003; Heupel and Simpfendorfer 2005; Guttridge et al. 2009a). Some studies have identified group social recognition where sharks display subordinate behaviour to individuals which are larger or of another sex (Myrberg and Gruber 1974; Klimley and Nelson 1981). Recent studies on juvenile lemon sharks *Negaprion brevirostris* (Guttridge et al. 2009a) showed individual sharks prefer to associate with members of the same species and individuals of similar size. In another study some individual lemon sharks led more groups than others and the leaders were usually larger than followers (Guttridge et al. 2011). All these studies appear to establish other complex issues being involved in group-joining decisions beyond recognition of species: individuals within a group are aware of the size of other members, and there may be deference to individuals of a greater size.

A recent review of shark agonistic behaviour by Martin (2007) showed that there are many common elements of behaviour in the displays of a variety of families of shark (Myrberg and Gruber 1974; Klimley 1985; Ritter and Godknecht 2000) which are typically motivated by self-defense. The agonistic displays are readily distinguishable from normal swimming or 'pseudo displays' caused by sharksucker irritation. A different style of agonistic display is the 'corkscrewing' of scalloped hammerheads *Sphyrna lewini*, which is made to displace others from, or retain a central position within the school (Klimley and Nelson 1981) and may be analogous to the 'head off' display by mantas described later in this Chapter. Myrberg (1991) proposed that fin markings in many sharks may serve as species recognition badges or to accentuate intraspecific or conspecific social signals and it is likely that mantas also use bold visual signals in a similar manner. Individual recognition has not been described in any elasmobranch however it has been described in teleost fish (Dugatkin and Wilson 1993; Griffiths and Ward 2006) and is expected from sharks and rays, some of which have greater cognitive abilities due to their larger brains (Ari 2011).

Aggregation behaviour appears common in other large planktivorous elasmobranchs. Aggregations of basking sharks *Cetorhinus maximus* and whale sharks *Rhincodon typus* may occur where they forage in rich prey patches and the aggregating may lead to courtship and mating (Sims et al. 2000; Hueter et al. 2008) but little investigation into social organisation of these species has been undertaken. High food abundance benefits

females, enabling them to mature at a larger size and aiding reproductive fecundity. The schooling behaviour of female scalloped hammerhead sharks *Sphyrna lewini* may be a refuging behaviour (Sims 2003), but appears to occur for courtship and mating purposes; with the largest most reproductively capable females competing to maintain position in the centre of the school via agonistic display to retain the most favorable mating status (Klimley and Nelson 1981; Klimley 1983; Klimley 1987). Ritualized agonistic interactions between mantas attending cleaning stations are regularly observed (Kitchen-Wheeler 2008). The displays are more fully described in this Chapter and discussed in the context of animal display behaviour and previously described shark behaviour.

Social networks have not been investigated in elasmobranchs, however studies of bottlenose dolphins *Tursiops truncatus* by Lusseau (2003) and guppies *Poecilia reticulata* by Croft et al. (2004) suggest that wild animal populations are characterised by non-random social network structure. Very little is known about factors underlying this structure e.g. sex or size, but Croft et al. (2009) showed that behavioural traits (boldness and shyness) strongly influenced social fine structure.

Sightings of individual *Manta alfredi* in North Male were investigated to identify whether there is any evidence of a social network and to identify whether there are trends in sociality from size and sex.

9.3 Method

9.3.1 *Social network investigation*

To investigate group structure and social network, sightings of North Male atoll based mantas were analysed using the Access database. Analysis was restricted to mantas sighted ≥ 5 times and only North Male atoll data included an adequate sample size (n = 84) of such mantas, which is a relatively small number. This sample of mantas included individuals first sighted from November 2001 until February 2007, and includes sightings until November 2010 and is representative of all known mantas from the atoll. Females were slightly over-represented in this sample (73.8% vs. 63.8% entire database) but there were sufficient males included in the study that conclusions on the differences in social behaviour of either sex could be drawn. Sightings of these mantas were analysed to determine an index of association (IA) between pairs of individuals based on Cairns and Schwager (1987).

$IA = X/(X + Y_a + Y_b)$

Where *X* is the number of times a pair of animals (*a* and *b*) were observed together, *Ya* is the number of times *a* was observed, but not *b*; and *Yb* is the number of times *b* was observed, but not *a*.

The analysis was made of sightings of these 84 mantas. Each manta's sightings data were compared with all other (83) mantas sightings and an individual IA for the relationship between each pair of mantas determined (potential range 0-1, 0 = never seen together, 1= always seen together). A mean IA for each manta was also calculated from the index of associations with all other mantas where a score was obtained (i.e. zero scores were excluded from the analysis). Data were not specific enough to determine precisely whether mantas were sighted in close company or not; but as the majority of data (>90%) were of sightings from single one hour surveys conducted on the date recorded, the mantas could be considered contemporary as both would be in the close vicinity of the cleaning station in order for both be to be reported within the hour. Instances of two different cleaning stations being surveyed in one day were very low (4) and these were accounted for by checking the location where the surveys were conducted. A small number of North Male atoll based mantas were sighted in other atolls and those records were included in the evaluation (i.e. the pair of mantas were seen together, or not). Two sets of results were obtained, an IA representing the relationship between a pair of mantas (0= no relationship, 1= always together so strong relationship) and an individual manta's mean IA obtained as an average of all the pair results scored for that manta excluding any zero scores. In the latter this is the mean result only where relationships exist so a manta might have a high mean IA but only have strong relationships with a low number of other mantas, or a low mean IA caused by having lots of relationships with different mantas. The results were searched for pairs of mantas sharing a high IA (> one SD above the mean) and for individuals with either high or low mean IA (> one SD above the mean IA, < one SD below the mean IA) to identify individuals with strongest and weakest associations which could them be investigated to identify any patterns in the composition of these sub-groups which might explain the difference in IA from variations in physical or behavioural traits.

9.3.2 *Investigation of group behaviours: arrivals and departures at cleaning station*

Mantas may arrive and depart from a cleaning station singly or as part of a group. Mantas which arrive solo may then depart as a group or vice versa. To determine

whether mantas form fixed groups during a short period of time i.e. a day, the arrival and departure of mantas to a cleaning station was investigated for mantas arriving in groups or singly and mantas departing in groups or singly. All timings are within 5 minute blocks so mantas arriving or departing within these blocks were considered contemporary. If there were significant numbers of mantas arriving and departing as fixed groups, this might be considered evidence that mantas form fixed groups during daily activities.

The dataset investigated in Chapter 7 (the continuous surveys on four dates at Lankan Reef) reported the arrival and departure time for each identified manta (the graph of each manta's attendances for each date is reported in Appendix C). The data were analysed for the times of arrivals and departures for each manta to determine whether they arrived and departed as part of a group, or individually. If there was evidence of group behaviour, the composition of the group could be further investigated by identifying previous and subsequent dates when members of the group were reported at Lankan cleaning station to determine whether the other members of the group were seen or not. Repeat sightings of a group on different dates would suggest that groups exist beyond a day.

9.3.3 *Observations of agonistic behaviour to determine dominance*

Specific examples of a common agonistic behaviour referred to here as a "head-off" have been captured on video during observations. Sequences of stills from video were analysed and behaviours described. Other examples of agonistic behaviour by mantas are also described.

9.4 Results

9.4.1 *Social network investigation*

The results Table for this investigation is 86 columns by 86 rows and cannot be presented in an A4 format as it is too large. A copy of the Excel file is available from the author on request. The results for each manta are summarised in Figure 9.1.

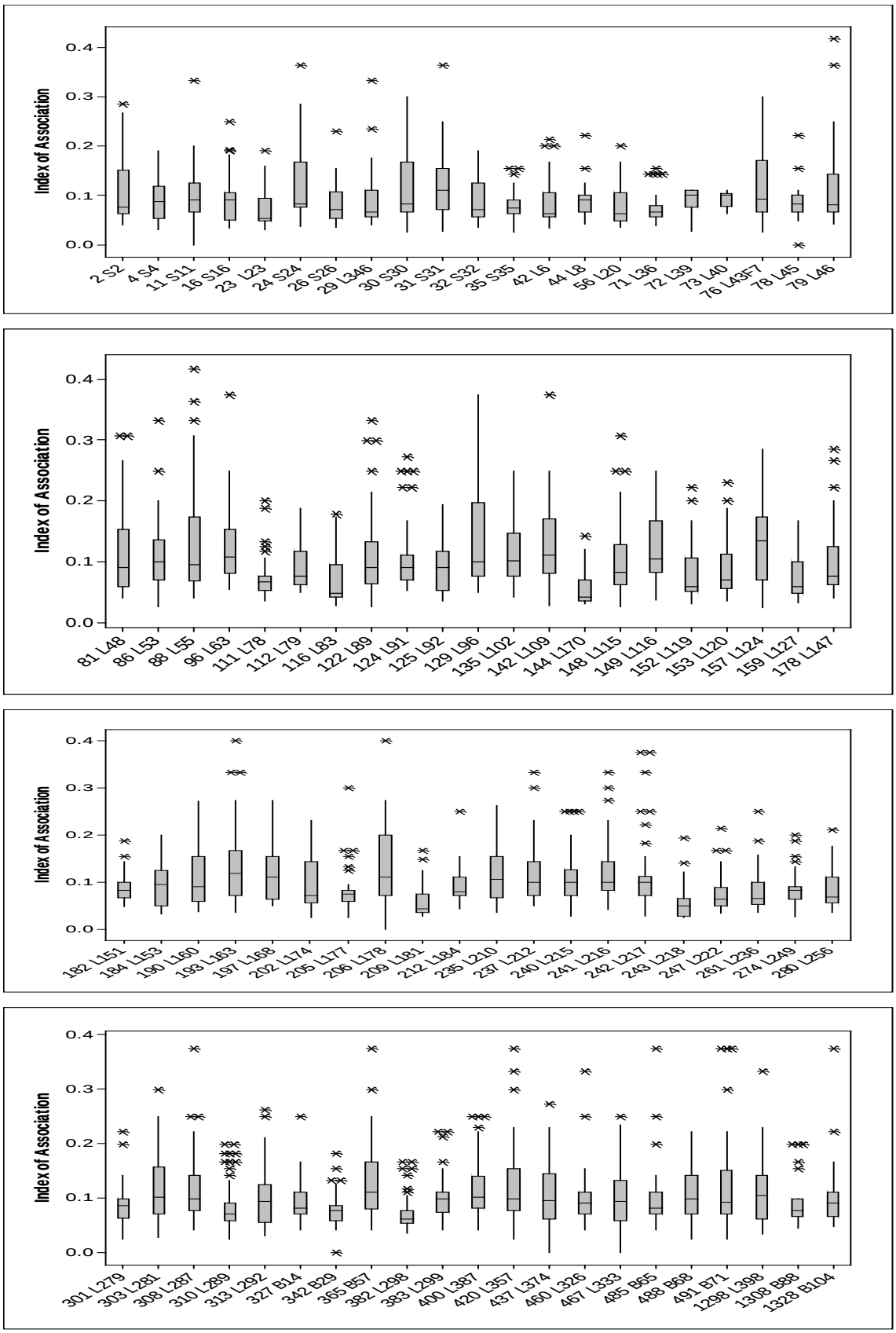


Figure 9.1 Boxplots of indeces of associations (IA) for each of the 84 individual mantas (zero results not included). The boxplot shows the range of values for all the indices for each individual manta with other individuals. High results (high outliers) suggest a relatively strong relationship between the manta identified and another. The majority of IA results were around 0.1.

The sample of 84 (62 female, 22 male) mantas were individually sighted on 5-33 occasions. 4398 relationships were discovered (index of association (IA) > 0) and summarised in Figure 9.1. 2574 pairs had no relationship (IA = 0). IA between pairs of mantas ranged from 0 (never sighted together) to 0.417 (the high outliers in Figure 9.1).

Excluding zero reports, the average individual mantas' mean IA was 0.1004 (SD ± 0.0195) (The distribution of the means IAs for each individual across each individual, Figure 9.2). Average female mean IA was 0.0978 (SD ± 0.0197), average male mean IA was 0.1077 (SD ± 0.0173). The distribution of female and male mean IA were normal (Anderson–Darling test for normality: AD = 0.332, *P* = 0.505: AD = 0.368, *P* = 0.40, respectively). The female and male results were compared using a one-way ANOVA.

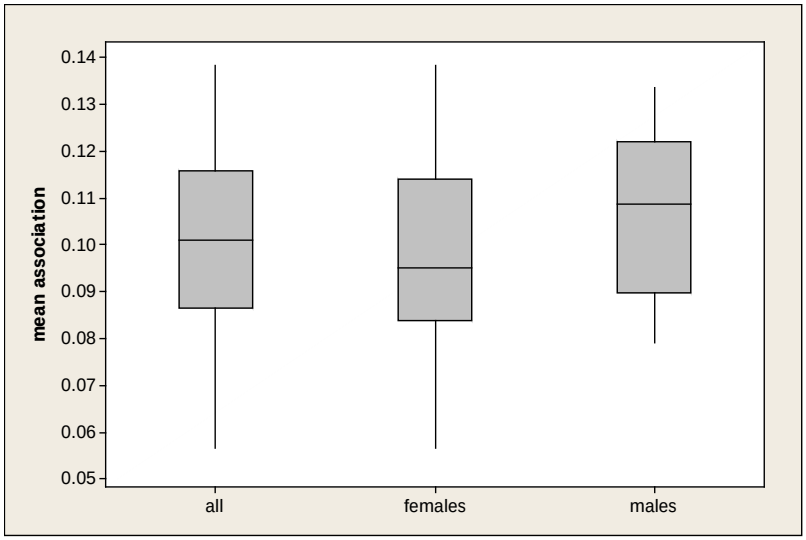


Figure 9.2 Boxplot of mean indeces of associations (IA) for 84 (all) mantas in North Male atoll which were seen 5 or more times. When separated into males and females and compared using a one-way ANOVA the average female mean IA was significantly lower than the average male mean IA: F = 4.35, *P* = 0.04.

The average female mean IA was significantly lower than the average male mean IA: F = 4.35, *P* = 0.04. The number of mantas (N) an individual had been sighted with (from the sample of 84) ranged from 8 (9.64% sample) to 76 (91.57% sample), mean 52.36 (SD ± 13.64) (Figure 9.3). A positive correlation was observed between individual mean IA and N (Pearson correlation = 0.316, *P* = 0.003). This suggests that the larger the number of mantas associated with an individual, the stronger the relationship with each one. A large, negative correlation between individual mean IA and the number of times the individual was sighted was also observed (Pearson correlation = -0.569, *P* <

0.001). This result suggests that the more frequently sighted mantas have weaker relationships with other mantas.

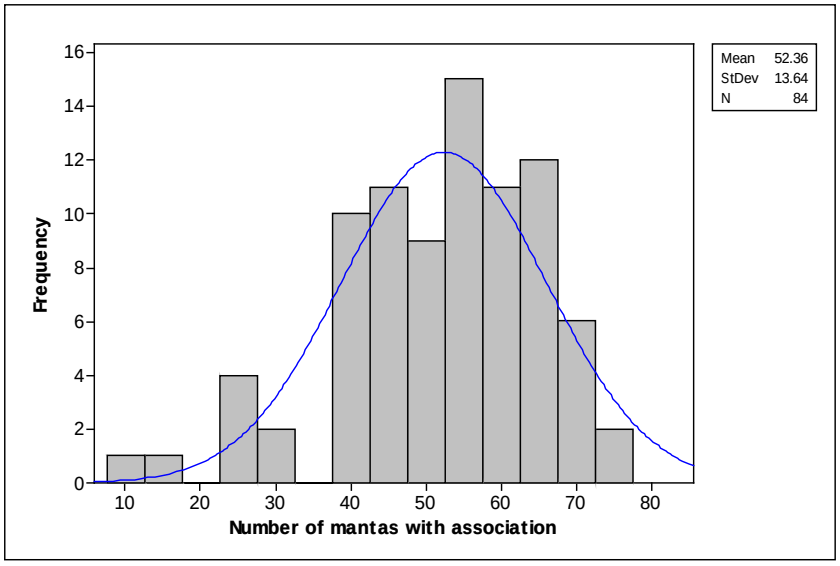


Figure 9.3 Histogram (with normal curve) to show the number of mantas associated for each of the sample of 84 mantas.

Mean sizes of mantas of different sex were significantly different (Chapter 4) so analysis for the effect of size was conducted by separating sexes. There was no correlation for size and IA for females or males.

Mantas scoring high or low mean IA (one-SD above or below mean) were further investigated (Table 9.1). Although more males appear in the high IA group compared to the low IA group the proportions are not statistically different. The sizes (DW) of the two groups were investigated using *t*-test to see if the mean DW of each group was the same. Mantas in the high mean IA group were significantly smaller than mantas in the low mean IA group (t = -2.74, P = 0.012, DF = 21) suggesting that smaller mantas were likely to have stronger relationships with other mantas than larger mantas. There was a correlation of mean IA and number of mantas associated with N; this was tested to see if there was a significant difference between the mean values of N for the high and low mean IA mantas. Mantas with high mean IA associated with significantly **larger** numbers of mantas N (t = 2.44, P = 0.027). This result supports the correlation of mean IA and number of mantas associated with, as expected for these mantas at the extremes of the sample. The number of sightings was compared for these groups and the high mean IA group were seen significantly less frequently than the low mean IA group

(t = -3.81, P = 0.003). These results suggest that mantas which are seen most frequently are more likely to be seen singly or on the same day as smaller numbers of mantas.

Manta ID	Mean IA (high group)	Gender	Number of mantas associated with (N)	No. of sightings	Manta ID	Mean IA (low group)	Gender	Number of mantas associated with (N)	No. of sightings
88 L55	0.133	M	61	7	23 L23	0.075	F	63	15
96 L63	0.126	M	44	5	35 S35	0.079	M	47	7
129 L96	0.136	F	56	6	56 L20	0.078	F	27	12
157 L124	0.134	F	68	10	71 L36	0.074	F	41	11
193 L163	0.136	F	70	9	111 L78	0.073	F	49	10
206 L178	0.133	M	62	5	116 L83	0.072	F	63	17
241 L216	0.122	F	69	6	144 L170	0.057	F	42	15
303 L281	0.133	M	62	5	159 L127	0.077	F	28	12
365 B57	0.131	M	54	5	209 L181	0.059	F	55	19
400 L387	0.121	M	56	5	243 L218	0.057	F	73	33
420 L357	0.120	F	57	6	265 L240	0.070	F	25	9
491 B71	0.126	F	48	6	382 L298	0.074	F	48	9

Table 9.1 North Male based mantas with high or low mean index of association (IA)

From this study, the mean IA for a manta could be used as a measure of sociability as IA correlated with number of mantas associated with, e.g. a manta with a high IA was likely to be sighted with more mantas i.e. in a group, but was likely to be sighted less frequently. The most frequently sighted mantas had a low mean IA, and were most likely to be seen singly or in smaller groups.

9.4.2 *Group behaviours investigation: arrivals and departures to cleaning station*

Groups attending cleaning stations consisted of all females, all males or mixed sex groups. Groups consisted of adults and juveniles of both sexes, although females were significantly more common than males (sections 4.3.2 and 4.3.3). All four day observations at Lankan showed new mantas arriving and departing throughout the day with a few returning several times. Some mantas arrived and departed as groups but there was no case of a group of mantas arriving, being cleaned and then returning in the

same group later on. Activity varied on different days but it was more common (78.0%, Table 9.2) for mantas to arrive and depart as part of a group (or sub group) than arrive and depart individually on these days. Investigation of individuals' IA for particular groups did not indicate patterns of visitation as a group. For example, on 8.8.2007 12 mantas were observed arriving together to the cleaning station at Lankan; of these, only five had been seen on four or more other occasions. The relationships (IA) between these five mantas are represented by the thickness of the lines shown in Figure 9.4. The largest IA is between 142 L109 and 206 L178 (0.25), the smallest between 310 L289 and 488 B68 (0.077) the mean for this grouping is 0.138 indicating a low level of relationship not suggestive of a "group". Two members of this group appear in the high mean IA mantas listed in Table 9.1.

Description of activity	17.7.2006	26.7.2006	31.7.2007	8.8.2007	Total each activity (%)
AG DG SG	5	36	14	49	104 (36.4)
AG DG SuG	10	10	6	8	34 (11.9)
AG DG DiG	8	32	14	31	85 (29.7)
AG DI	8	3	0	6	17 (5.9)
AI DG	9	3	1	5	18 (6.3)
AI DI	4	4	14	6	28 (9.8)
Total (day)	44	88	51	105	286

Table 9.2 Number of mantas observed arriving as a group (AG) or as individuals (AI) and departing as same group (DG SG), or departing as a sub group (SuG), or different group (DiG), or departing as individuals (DI)

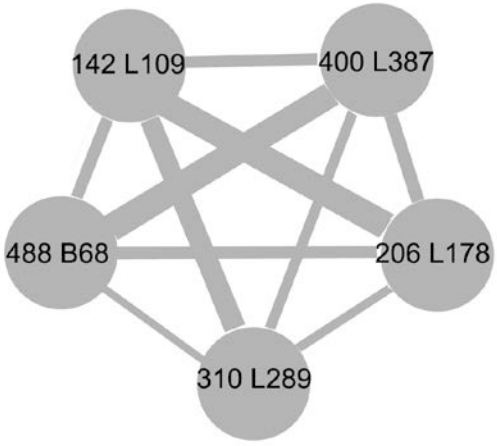


Figure 9.4 Sociogram of relationships between five mantas seen arriving together and departing together on 8.8.2007. The thickness of lines represents index of associations and is strongest between 488 B68 and 400 L387 (0.222)

9.4.3 *Description of agonistic behaviour: 'head-off' display*

The display sequence referred to as a 'head-off' was commonly observed when there were more than two mantas present and may be a display to establish dominance as the loser consistently swims away. The displays were observed at all cleaning stations and the estimated rate of occurrence is approximately one interaction per hour survey. The incident reported (Figure 9.5) took place at Lankan on 23.10.2003.

The sequence commenced with two animals swimming towards each other horizontally whilst facing each other (Figure 9.5a). Once the heads were within a metre distance, the mantas swam upwards, abdomen to abdomen (Figures 9.5 b and c) whilst appearing to compare the size of their extended wings. The eyes are located on the side of the head so the extension of the wings would be visible. The larger manta held position whilst the smaller manta then started to swim sideways and downwards. In Figure 9.4 d the manta in the foreground was larger and won, the manta in the background (the loser) started to swim downwards and away. The loser always appears to swim away below the level of the winner (Figures 9.4 e and f). I have termed this behaviour a head-off as the competition starts with the mantas swimming towards each other **head** to head, and ends with one manta swimming away (swimming-**off**). Slight variations were observed, typically when the losing manta swam away without the full abdomen to abdomen part of the routine being required (Figure 9.5). The head-off routine was sometimes performed by unlikely contenders in that one manta was clearly smaller than the other. The larger manta consistently wins the head-off and it is likely that the display is performed in order to settle a dispute over priority access to the cleaning station and is thus a display to establish dominance.

Only one incident was observed when a manta apparently used force to see off an opponent. Manta 178 L147 and one other manta were already established on the cleaning station at Lankan when manta 243 L218 (who incidentally, is also the victor in Figure 9.5) swam over the main cleaning area. Manta 243 L218 made a sideways swimming motion angled at about 45º into manta 178 L147 and then made three forceful pushes into her flanks causing her to be moved off to the side. Manta 178 L147 made an attempt to recover her position above the cleaning station but manta 243 L218 made a final push and manta 178 L147 swam away. The incident was witnessed by several very experienced manta watchers who agreed that the bumping action appeared aggressive. Both mantas had an estimated DW of 4.0 m. The mantas have been sighted together subsequently on three other occasions and no agonistic behaviour was

observed. The bumping behaviour was unusual as it is the only example of apparently aggressive, physical contact between mantas except during mating. The aggressor (243 L218) is listed in Table 9.1 with a low mean IA (0.057). The identities of other mantas winning head-offs were not recorded and the winning manta in Figure 9.6 has only ever been seen three times so was excluded from the IA study.

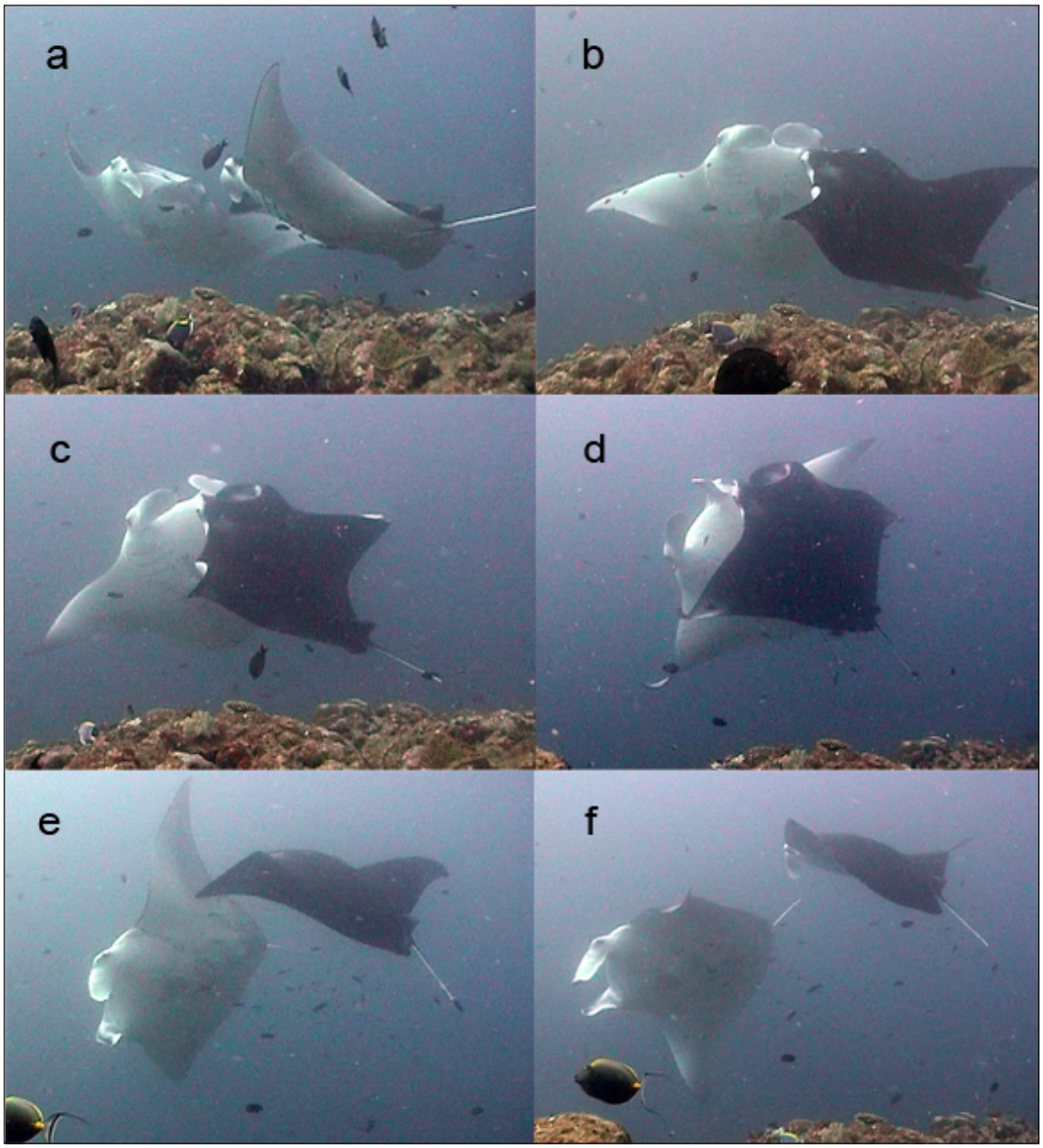


Figure 9.5 a to f. Precedence at a cleaning station may be settled by mantas swimming towards each other (a); then swimming upwards, abdomen to abdomen (b) and (c) so as to allow comparison of size via the wing tips. The larger manta (foreground) won the competition and the smaller manta swam away sideways and downwards (d)(e)(f). I have termed this display a head-off.

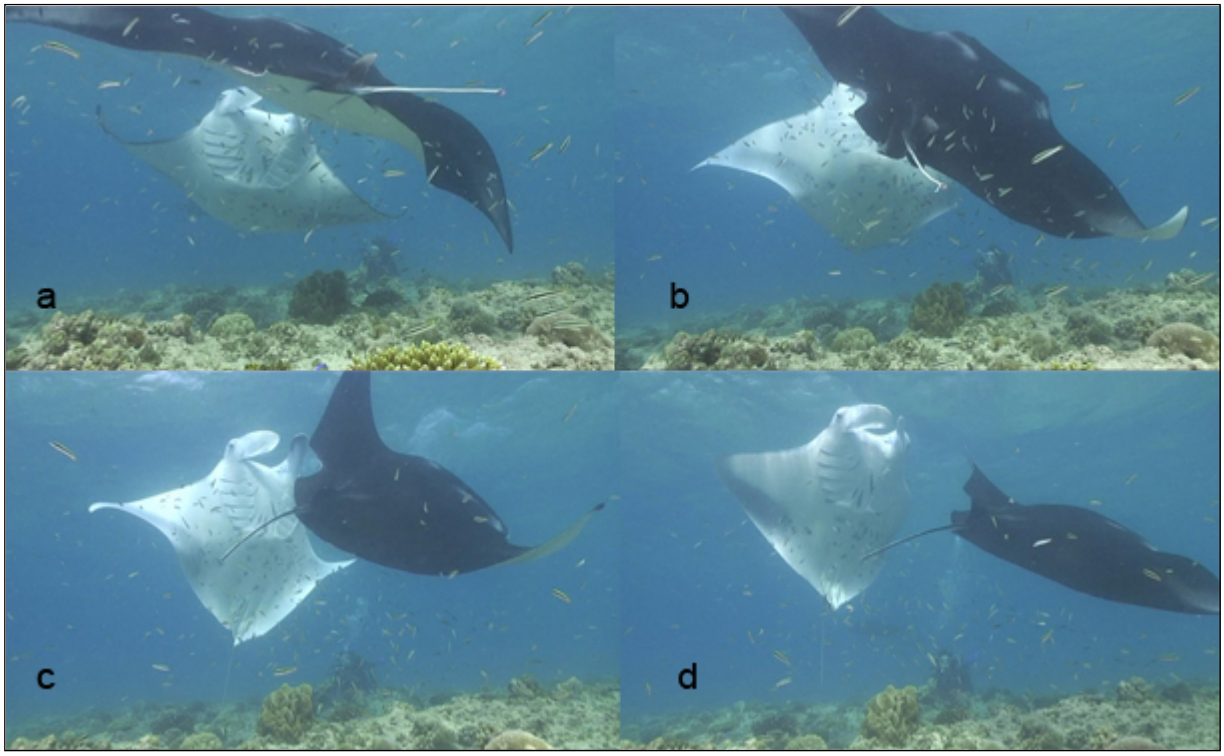


Figure 9.6 A truncated version of the head-off display. (a) Manta in background, maintained a dominant stance towards the approaching manta (foreground). In (b) the submissive manta (foreground) did not continue to swim at the dominant manta but swam off to the right (c) and then swam down to confirm its submissive status (d)

9.5 Discussion

9.5.1 *Social network investigation and group behaviour*

In the absence of any published research on manta relationships this study has been useful in establishing some measures for the relationships between mantas. The mean IA between any pair of mantas was 0.1004 (SD±0.0195) thus any pair of mantas with a much higher IA (>0.3) suggests a relationship between those individuals as each would have had to have been sighted a minimum of 5 times to have been included in this study and the IA of >0.3 suggests they would have been reported “together” during approx. 50% of these sightings. That the most frequently sighted mantas had the lowest mean IAs suggests that these high results may just be caused by chance. Presently we do not understand manta movements and behaviours sufficiently to determine what level of IA suggests a strong relationship. Overall, the moderate mean IA for the majority of pairings does not support the hypothesis that mantas form stable groups. Instead, whilst some individuals show stronger relationships with others, there appears to be a low level of association overall. From population studies carried out in North Male atoll and around Lankan and Boduhithi (Chapter 6), the population numbers

around a cleaning station may be 130-810 mantas. Co-operative feeding behaviour of aggregations of 10-150+ mantas is discussed in Chapter 8 and the maximum number of mantas to have been sighted at a cleaning event was over 100 mantas so the size of a manta social group may be much greater than suggested by the size of typical groups attending at cleaning stations (2-15) i.e. social groups may consist of 100+ mantas and only sub-groups of 2-15 are commonly seen at cleaning stations. The low mean IA, the relatively small range of the mean IA and high numbers of mantas (mean 52.36 mantas, equivalent to 63% of sample of 84 analysed) with which associations are known for each manta sampled, further supports the new hypothesis that stable groups are not typically formed, only loose associations from a local population.

Croft et al. (2009) proposed that social fine structure was strongly influenced by behavioural trait with shy fish having more and stronger connections than bold fish (identified from predator inspection tendency). Of mantas with the highest and lowest mean IA (>one SD above or below the mean), the mantas with low mean IA included the most frequently sighted mantas: Butterfly 243 L218, Bubbles 116 L83, Brenda 209 L181, Comet 144 L170 and Bunny 23 L23. These individuals are famous for their interactions with divers, seeming fearless of humans and are all large, mature females. Butterfly, Bubbles and Brenda are frequently seen performing 'head-off' displays. These females might be considered as having a bold behavioural trait. It appears that behavioural trait may have an influence on manta social structure and should be further investigated. The high IA group were significantly smaller (mean DW) than the low IA group. If the correlation of IA and number of mantas associated with (N) are also taken into consideration, these results suggest that smaller mantas are likely to form larger groups to visit the cleaning station as would be expected from animals more likely to show the shy behavioural trait as they are younger (smaller) and lower in the hierarchy.

9.5.2 *Head-off display*

For behaviours to be considered ritualised, they should be formalised, socially prescribed and symbolic (Winthrop 1991). Displays may evolve from non-communicative source behaviours which are ritualised and then shaped by natural selection (Tinbergen 1952). Ritualised behaviour was suggested to prevent harm: the posturing in threat displays allowing assessment of combatants likely success, without combat actually taking place (Lorenz 1965). Agonistic displays in sharks have been rarely reported (Bres 1993). The grey reef shark *Carcharhinus amblyrhynchos* is one of

the few shark species known to make agonistic displays. They exhibit a distinctive, highly stereotyped "hunch display", named for the characteristic raised-snout, arched-back, fins-down pose (which resembles an exaggerated shrug) section of a four-posture display. The "hunch" segment in the display is suggested to have derived from movements involved with subjugating prey (Barlow 1974). Zahavi (1993) predicted that signalling of an animals' state or motivation is likely to be accurate if the signalling is costly i.e. it handicaps the signaller in some way.

In the "head-off" display the same sequence of behaviours is performed each time, thus it might be considered formalised. A "head-off" would meet the criteria of a display because the head-up position is symbolic and likely to have derived from the initial stages of a partial somersault. The somersault might be used in avoidance (as a rapid turn to change direction), but is also used when feeding (Chapter 8), and is costly to the manta(s) displaying as the head up position makes the animal vulnerable to an abdominal attack by another animal. Additionally, no contact or injury is caused; which also suggests the behaviour is symbolic, as are other shark agonistic displays described by Martin (2007). The head-off display should be considered agonistic rather than avoidance behaviour because the loser either swims away defeated, or remains in the vicinity but behaves in a submissive manner around the winner. The display is probably used to establish dominance amongst mantas over access to resource, the resource being disputed in these examples is access to the cleaning station and dominance is established via comparison of size. If a vertebrate is threatened, it often displays its flank (Carpenter 1978; Hinde 1982) in an attempt to show the predator its size and discourage further attack. Flank displaying was putatively reported in sand tiger sharks *Carcharias taurus,* white sharks *Carcharadon carcharias,* silver tip sharks *Carcharinus albimarginatus* and bull sharks *C. leucas* (amongst others) by Martin (2007). Back arching, tilting and rolling movements were also made by sharks in self-defence to make the defender appear as large as possible (Martin 2007). The head-off display may also derive from self-defence behaviour as when a manta is threatened by a predator (or as observed several times when a diver was too close), a manta rears up to show the ventral side which is the same posture as adopted by a manta in a head-off display. Head-off displays appear to establish size as the primary measure of social dominance within a group of mantas i.e. largest is dominant. The two mantas in Figure 9.2 have been observed at Lankan together on three occasions and an agonistic interaction was only observed during one observation, suggesting that the individual

mantas may recall the result of previous disputes and lower ranking mantas continue to respect dominant ones for an extended period time (weeks or months). This is only one example but the identities of mantas involved and the result of future head-offs can be recorded to identify if later displays are observed between that pair of mantas. The importance of the head-off display is that it establishes that, as in other elasmobranch agonism situations, largest is most important; but unlike other species (including the scalloped hammerhead shark, *Sphyrna lewini* (Seifert 2001)), size, and thus dominance can be ascertained without physical contact and possible damage to an individual which is beneficial to the population as a whole. The rarity of physical force between mantas should be noted. There is only one other recorded example of a manta using physical force on another and that was by the captive male manta at Okinawa aquarium towards its offspring when only a few days old (Weiss 2007) where the male forced the baby into the sides of the aquarium and it died after 5 days from its injuries. My data suggest that physical violence is rare between mantas (one incident of "jostling" in nearly 400 hours of observation), but agonistic behaviour may be reduced when mantas become wary of human presence.

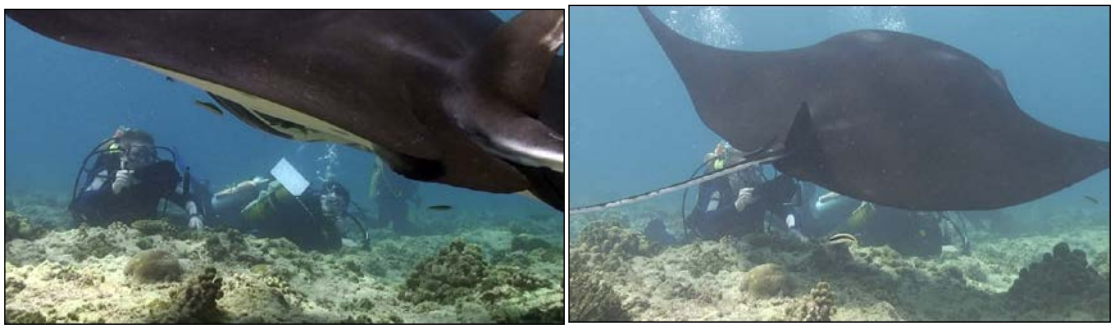


Figure 9.7 Diver observers are briefed to remain low on reef to avoid threatening mantas (left). A manta investigates the divers very closely and swims over their heads (right), perhaps to establish her dominance.

Scuba divers are encouraged to adopt certain behaviours in order to achieve extended observation at cleaning stations (Harwood and Bryning 1998). The recommendation is to remain close to the reef and away from the cleaning station. This behaviour is submissive in that the diver stays still and low so there is reduced potential for perceived threat to any manta present. Submissive behaviour by divers may lead to investigation by mantas which may be simple curiosity and also may be mantas performing dominance display by swimming over the heads of the divers. An example is shown in Figure 9.7. At first this appears to just be a very close pass by the manta however the sweep over the heads of the divers was deliberate and unnecessary. This

may be another variety of agonistic display behaviour and should be investigated further.

9.6 Next steps

The investigation of index of associations between mantas produced measures of relationship between 84 of the most frequently sighted North Male mantas and more analysis of the results to identify sub-groups could be made with computer modelling. The most important mantas to investigate would be sub-groups with strong mean IAs. These should be investigated to determine whether the associations exist over time. Mantas with long histories and many sightings could also be investigated for patterns of associations with other mantas e.g. whether only seen together at certain times of year. The investigation of exceptions i.e. mantas never seen together, within the mantas seen very frequently should also be conducted.

This preliminary study of social behaviour in *M. alfredi* describes the stereotypical agonistic display called a head-off. The extent of its use and investigation into other possible agonistic behaviours are the obvious subjects for future study. The recording of the combatants and result of all future head-offs would be useful in order to produce a hierarchy table as it might be possible to determine whether the result of a head-off is remembered by the mantas, and if so, for how long. There are other social interactions conducted by mantas which are commonly observed at cleaning stations but not reported in this Chapter. As with the head-off, more repetitions of the behaviours need to be recorded so that analysis of the behaviours (rather than just a description) can be made.

Chapter 10. Reproductive behaviour of Alfred mantas (*Manta alfredi*) in the Maldives

10.1 Abstract

A strong female bias (almost 2:1 overall) with significant variation between sites was observed in this study. The study sites were manta cleaning areas so the gender mix discussed here represents that seen at the cleaning stations surveyed. Studies of captive Alfred mantas suggest a normal pregnancy of 12-13 months durations. This was supported from observations of wild mantas in this study. There appeared to be two mating seasons in the Maldives, around October-November and February-March. This hypothesis was supported by results showing peaks in numbers of pregnant mantas being observed in July and August, prior to parturition and the autumn mating season; and a protracted period of sightings of pregnant mantas likely to result in parturition being February-March, coinciding with the second mating season. The proposed mating seasons would coincide with the latter period of the prevalent monsoon, and it was suggested that the peaks in mating activity were caused by the high productivity also observed at this time nearby the cleaning stations visited by the mantas. The high productivity would attract mantas to congregate in these areas, principally to feed. Two types of courtship activity were described in this population of *Manta alfredi*: "shadowing" and chasing. "Shadowing" is where the male positions himself parallel to, and above the female's dorsal surface whilst stimulating her dorsal surface using his cephalic fins. The behaviour had not been described scientifically before. Shadowing courtship was observed throughout the year and suggests that mating may also occur at any time in the year. It appeared that female mantas appear choose which partners they mated with following both forms of courtship. The distribution of pectoral fin scars, caused by the male biting on the female during mating, was highly biased to the left wing indicating a strong lateralized behavioural trait. Whilst it appears that *Manta alfredi* may conceive shortly after parturition in the wild, 24 month (or longer) cycles were more commonly reported. It was proposed that mantas conceive much less frequently than every 24 months based on observations of regularly sighted females over eight years in this study. As only one pup is apparently born from each pregnancy there are worrying implications for conservation of the species in areas where there is a targeted fishery.

10.2 Introduction

The reproductive behaviour of *Manta alfredi* has, until recently, been poorly studied. The reproductive cycle was unknown, and there was only one report of mating behaviour (Yano et al. 1999b), which appeared to be of *Manta birostris* from visual assessment of the photographs. The literature on reproductive anatomy and behaviour of *Manta* species was reviewed in section 2.7. This Chapter reports on studies of the gender mix and size; mating and gestation cycles; and observations of courtship and other reproductive behaviours of *M. alfredi* in the Maldives.

There was a recent study of the reproductive ecology of *M. alfredi* in Mozambique by Marshall and Bennett (2010b). The study was contemporaneous (May 2003 to March 2008) to this study but the results did not come to my attention until after the Maldives study was completed. Independent investigations on mating scars, reproductive periodicity and courtship behaviours were made. The Mozambique study proposed that the region encompassing the study sites was a mating ground for *M. alfredi*. After the Mozambique report was published it was useful to compare results on gender mix, size and reproductive periodicity between the two studies. Comparisons are reported in the results section and discussed later in this Chapter.

A 1:1 primary sex ratio (at conception) would be expected in animals using sexual reproduction (Fisher 1930; Charnov 1982; Edwards 1998). Early observations from this study suggested that the sex ratio of males and females was unequal with the overall population being reported with a 1:1.8 male: female ratio as reported in Chapter 4. A high female bias was suggested to be connected with reproductive strategies by Marshall and Bennett (2010b). The sex ratio was investigated at site level in this study to determine where there were any differences in the populations of mantas visiting different sites. The size at sexual maturity in this species had been reported for a small number of individual *M. alfredi* of both sexes (White et al. 2006; Uchida et al. 2008). Although size (DW) of individual mantas were estimated in this study, data were available (from the Access database) on a large number of adults which might provide a useful mean size of sexual maturity for each sex.

Courtship and mating behaviours of mantas were regularly observed near cleaning stations in the Maldives and appeared to involve specific rituals which are described and discussed in this Chapter. Mating behaviour appeared to intensify during a period

of around four weeks during October to November and February-Match each year. The actual four-week period would vary slightly from year to year but it was anticipated that during this time chasing and breaching behaviours might be observed during these times, but are not seen outside of these periods. These periods became known locally as manta mating seasons and the phenomenon is investigated here. Two cycles from mating (conception assumed) to birth (two pregnancies) in a female *M. alfredi* in captivity were reported by Matsumoto and Uchida (2008). These suggested a gestation period of 12-13 months and that conception could occur immediately after parturition (consecutive pregnancy). With a likely 12-13 month gestation period, sightings of heavily pregnant mantas can be used to estimate when mating might have occurred. This information can be used to look for evidence of mating seasons. Sightings of pregnant mantas could also be used to determine whether consecutive pregnancy occurs within wild populations and investigate the frequency and periodicity of pregnancies from observations of frequently sighted adult females.

Female mantas were frequently sighted with scars on the left dorsal pectoral fin (wing) tip. Yano et al. (1999b) described male *M. birostris* attaching to the female during copulation by holding the left wing-tip. Other male elasmobranchs are known to attach to the female during mating, the behaviour being observed so frequently that it is reported in fish guides aimed at interested amateurs (Henneman 2001). I was especially familiar with the behaviour in grey reef sharks *Carcharhinus amblyrhynchos* and white tip reef sharks *Triaenodon obesus* from multiple observations of matings in the wild. A mating event involving multiple *T. obesus* was reported in (Tricas and Feuvre 1985). Following matings, the females bore scars in the area where the male shark attached (the gill-slits in the species of reef shark mentioned above) for periods of several months after the mating. The commonness of the markings on the left wing-tip of only female mantas led me to conclude that the marks were mating scars. Later knowledge of the study by Yano et al. (1999b) confirmed my observation. The attaching behaviour and identification of the left wing-tip scar as a mating scar were also recently reported by Marshall and Bennett (2010b) for *M. alfredi*. An investigation of wing-tip mating scars and other abrasion scars connected to mating was made.

10.3 Method

10.3.1 *Gender mix and size (DW) of adult population*

The Access database as of November 2007 containing the records of 1440 mantas was analysed for the gender mix ratio study reported in Chapter 4. Gender mix of the general population was reported in section 4.3.2. All data were for mantas sighted at cleaning stations. This data set was re-analysed in this study to identify whether there was any variation of gender mix and maturity with survey site. The data were separated by site and reported in table 10.1.

Sexual maturity can be assumed in females if they were clearly pregnant. Pregnancy was determined from obviously distended ventral and dorsal surfaces (Figure 10.1). Marshall and Bennett (2010b) also considered females with mating marks (section 10.3.3 in this study) to be mature, however this characteristic was not routinely recorded in this study until after 2008, so data were missing from the majority of female records.

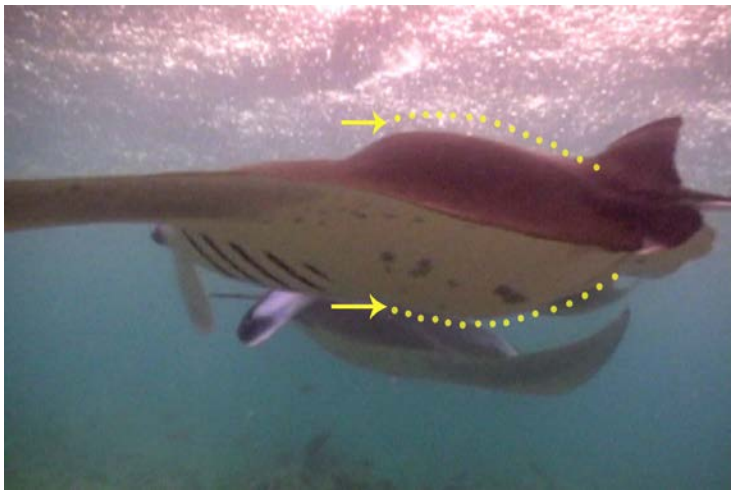


Figure 10.1 A heavily pregnant female manta with distended dorsal and ventral surfaces outlined in yellow. This manta is likely to be in the final trimester of pregnancy.

It was assumed that a mature male with enlarged and calcified claspers (Pratt and Carrier 2005) (Figure 10.2b) or claspers extending beyond the posterior edge of the pelvic fins was ready to mate (following Walker (2005) and White et al. (2006)). These features can be determined from visual assessment of the claspers and genital area either during observation, or from analysis of photographs. These features do not confirm that the male is ready to mate; only that it is likely to be mature. Males with partially extending claspers, lacking calcification (Figure10.2a) were considered sub-adult; and males with partially formed claspers, or not extending beyond the posterior edge of the pelvic fin were considered juvenile, as in Marshall and Bennett

(2010b). All database photographs of males were assessed to investigate the state of claspers and determine whether mature, sub-adult, juvenile or unknown (genitalia was missing from photograph or unclear).

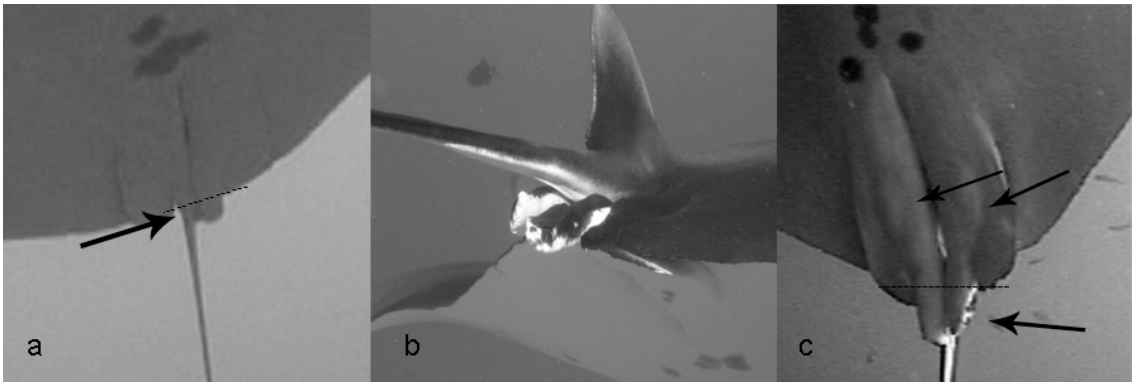


Figure 10.2 Male *Manta alfredi* genitalia: (a) sub-adult male (1398 L262) showing ends of claspers just extending the rear of the posterior edge of pelvic fins, but do not show clasper gland structure anterior to the claspers and appear smooth and un-calcified; (b) adult male showing calcified claspers seen from side, extending beyond pelvic fins and disc; (c) adult male (69 L34) showing enlarged clasper glands (arrowed top) anterior to the calcified claspers which extend well beyond the edge of the pelvic fins (arrowed bottom).

Notes in the comments section of the Access database on pregnancy in females, mating scars and courtship activity by females and males were also analysed. In other studies the minimum length where 50% of animals are mature (L_{50}) was used as a base measure of size of maturity. In this study the majority of females were not sighted pregnant, although they might have been mature. The majority of males which could be assessed from examination of the condition of the claspers were mature. The L_{50} method could not be used as the models require proportions of mature and non-mature individuals for each size to be ascertained (Corro-Espinosa et al. 2011; Zhu et al. 2011). In the case of females, their status could not be ascertained so no calculation was possible: in males, only 6 immature mantas were identified which was too small a proportion to provide a useful result. However, the evidence obtained from the samples was used to describe the mean size of known adults within the observed Maldives population.

10.3.2 *Periodicity of mating activity and pregnancy*

The comments sections of the Manta details form in the Access data base were analysed to identify all dates of individual female mantas being sighted pregnant. *M.*

alfredi are only visibly pregnant from ~month 8 of gestation onwards. Sequences of dates for each manta were investigated to determine whether dates were of sightings of a single pregnancy (e.g. dates within ~4 months during the period when visibly pregnant) or were indicative of several pregnancies (e.g. dates were from different years or more than eight months apart). If a female was reported pregnant in October and not pregnant in December of the same year it was assumed she had delivered in the interim. Knowing the gestation period to be 12-13 months (Matsumoto and Uchida 2008) it could be assumed she had conceived in October-December the previous year. For example, heavily pregnant manta (243 L218) sighted in October and early November 2005 and predicted to deliver in late November 2005, was likely was to have conceived during the previous October-November. Manta 542 M43 sighted pregnant in December 2006 could not have recently conceived, so the conception was likely in the previous February-March period and would be likely to deliver in February-March 2007 etc. The dates of sightings were analysed for all females ever reported pregnant to determine the number of times each manta was pregnant and the likely months of conception and delivery for each gestation period. When only a single date within a gestation period was recorded other facts were investigated to establish the likely cycle e.g. comments on mating activity to identify when conception may have occurred (courtship by males, new mating scars reported etc.); or whether the female appeared very heavily pregnant, suggesting she was close to delivery etc. It was assumed that fertilization occurred immediately after mating and that sperm was not stored for later fertilization (Pratt 1993). A significant variation in the number of mantas sighted pregnant with month would support the hypothesis that there are mating seasons in the Maldives.

If there are mating seasons then expected birth month can be predicted for a pregnancy. Based on a 12-13 month gestation, peak sightings of pregnant mantas should occur from around four months before the anniversary of conception, and continue up until a month after the anniversary. A visibly pregnant would be expected to deliver within the next four months: most likely the delivery would be during or slightly after the next mating season. This information can also be used to identify the likely month of conception for that pregnancy. Individual “case histories” of female mantas reported with more than one pregnancy were investigated to identify the probable length of time between pregnancies (periodicity).

10.3.3 *Observations of courtship and other reproductive behaviours*

Digital video and photographs of courtship behaviours were taken at Lankan, Boduhithi, Madivaru and Table Thila cleaning station sites. These were analysed to characterise common patterns of behaviour. Types of scars obtained during mating behaviour are described. Full descriptions of the observation method, site information and identification method are available in Chapters 3 and 4.

10.4 Results

10.4.1 *Gender mix and size of sexual maturity*

Of the 1422 *M. alfredi* for which the sex could be determined, 514 (36.1%) were recorded as male, 908 (63.9%) as female. A Chi-square test indicates a strong sex bias in the observed population ($\chi^2 = 109.2$, $P < 0.001$, DF = 1). A female bias was observed at all main survey sites ranging from 72.6% at Madivaru to 54.9% at Boduhithi (Table 10.1). Bias varied significantly between sites (Yates corrected $\chi^2 = 13.35$, $P = 0.012$, DF = 5).

112 individual females were observed pregnant. Their mean size (DW, by visual estimation) was 3.73 m (SD ± 0.354) with a range of 3.0 to 4.5 m. The only pregnant manta actually measured was 3.8 m DW. The mean DW of pregnant females was significantly greater than that of females as a whole (all females n = 870, mean DW 3.26 m (SD ± 0.513)) when compared using a 2-sample *t*-test ($t = 12.47$, $P < 0.001$, DF = 177). Adult males (n = 193) as assessed from database photographs of individuals showing large calcified claspers had a mean DW of 2.81 m (SD ± 0.38) and a range of 2.0 to 4.0 m DW. The mean DW of adult males were significantly greater than that of all males (n = 504, mean DW 2.69 m, (SD ± 0.407)), compared using a 2-sample *t*-test ($t = 3.66$, $P < 0.001$, DF = 370).

The size of mantas (separated by sex) at each survey site was analysed to determine the mean size for each site to see if there was a difference in the populations of mantas which visited the sites, i.e. whether sites had more or fewer adults of either sex than other sites (Table 10.1). When compared using a one-way ANOVA there was a significant variation in mean size of both males and females between sites (one-way ANOVA females: F= 8.62, $P < 0.001$, DF = 4; males: F = 11.20, P < 0.001, DF = 4). However males observed at Boduhithi, Lankan, Sunlight, Madivaru and Kalhahandi were of similar mean DW to the known all-adult group when compared using Tukey-

Kramer all pairwise comparison test indicating the majority of males sighted at these sites are likely to be mature. The estimated mean size of male mantas at Table Thila was significantly smaller than those at Boduhithi and Lankan and of the known all-adult group. This suggests that there were more immature males reported at Table Thila. It is difficult to draw conclusions from the other results. The mean DW of females reported from Boduhithi were the largest from all sites and on comparison using a Tukey-Kramer all pairwise comparison test were significantly larger than females at Lankan and Madivaru, but significantly smaller than all-adult females. This suggests that there are more mature females at Boduhithi than other sites, but the population visiting the site still comprises a mixture of adult and non-adult females.

Site	**Male count**	**% male**	**Mean DW±SD**	**Female count**	**% female**	**Mean DW±SD**	**Total**	**Males: Females**
Sunlight	11	29.7	2.57±0.47	26	70.23	3.31±0.63	37	1:2.4
Lankan	144	36.5	2.73±0.35	250	63.5	3.31±0.46	394	1.1.7
Boduhithi	105	44.1	2.86±0.37	128	54.9	3.47±0.39	233	1:1.2
Table thila	180	37.3	2.55±0.42	303	62.7	3.21±0.58	483	1:1.7
Kalhahandi	9	32.1	2.83±0.61	19	67.9	3.20±0.49	28	1:2.1
Madivaru	48	27.4	2.61±0.34	127	72.6	3.08±0.54	175	1:2.6
Total	**497**	**36.8**	**2.69±0.41**	**853**	**63.2**	**3.26±0.51**	**1350**	**1:1.7**

Table 10.1 Gender mix and mean size of mantas reported from main survey sites

10.3.2 *Periodicity of mating activity and pregnancy*

The sightings records for each of the 112 female mantas ever recorded as pregnant (SP group) were sorted to ascertain the month when the individual manta was sighted and reported in Table 10.2 when each manta was pregnant (A) and when not pregnant (B). For example, there were 8 of the 112 mantas sighted pregnant in January and 13 of the 112 mantas sighted not-pregnant in January. This was to identify months with the highest ratios of pregnant mantas within the SP group of 112 mantas which were known to be able to conceive. Each manta was only recorded once per month per year in the table. Sightings records for all female mantas (AF group) were also sorted to determine month of sighting (D) so that the percentage of pregnant mantas vs. all female mantas sighted could be calculated. The results are shown in Table 10.2 and Figure 10.3.

Month	Number of pregnant mantas each month (A)	Sightings of same set of mantas when not pregnant (B)	Sightings of same mantas when pregnant (A/A+B x100%)	Number of sightings all ♀ mantas (D)	% all ♀ pregnant (A/D x100%)
January	8	13	38.1	128	6.25
February	37	41	47.4	506	7.31
March	25	29	46.3	354	7.06
April	3	3	50.0	55	5.45
May	1	17	5.6	63	1.58
June	3	33	8.3	102	2.94
July	14	46	23.3	389	3.59
August	20	15	57.1	66	30.3
September	0	13	0	64	0
October	27	63	30.0	224	12.05
November	4	47	7.84	99	4.04
December	12	17	41.4	169	7.1

Table 10.2 Table to show numbers and percentages of pregnant females each month within the peer group of mantas ever-seen-pregnant, and of all females.

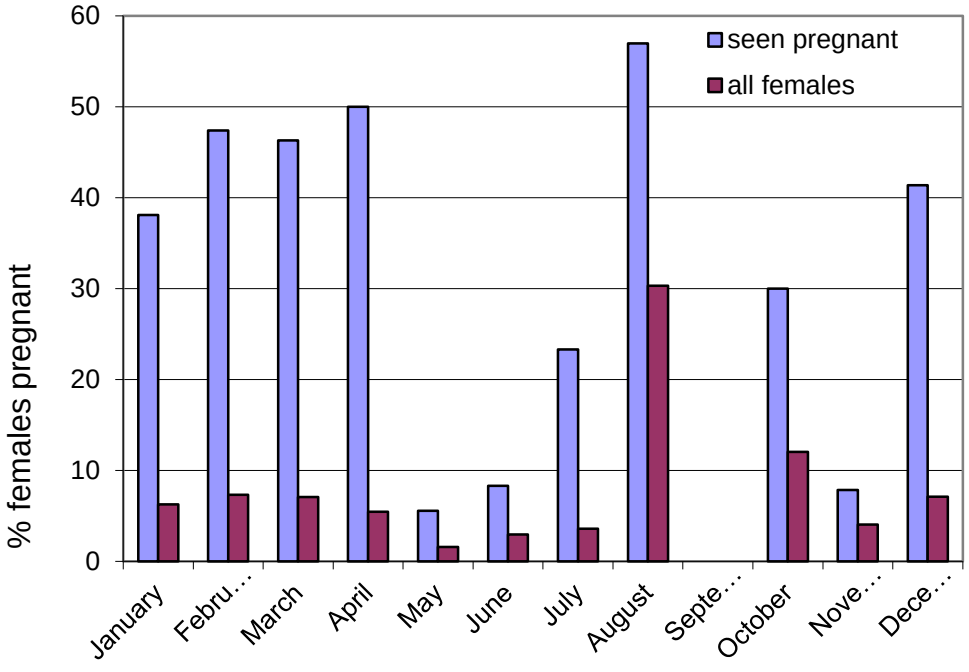


Figure 10.3 Graph of the data shown in Table 10.2 (columns 4 and 6) showing the percentage of females seen pregnant each month from the seen pregnant (SP group, blue bars) and percentage of pregnant amongst all female mantas (AF group, red bars). No mantas were reported pregnant in September.

Rayleigh tests on the two sets of results were performed to determine whether percentage of pregnancies were significantly non-uniform with respect to time of year. The Rayleigh tests of percentage of mantas ever seen pregnant (SP), and percentage of all females ever seen pregnant (AF) distributed throughout a year showed significant non-uniform distribution around the year. For the SP group the mean vector was 7.56 months (suggesting July); critical value was 27.42 when compared with χ^2 statistic with 2 DF where 13.82 is significant at $P < 0.001$. For the AF group the mean vector was 8.54 months (suggesting August) and critical value was 7.782 when compared with χ^2 statistic with 2 DF where 5.99 is significant at $P < 0.05$. These results indicate July and August as the months with peak sightings of pregnant mantas. These results provide evidence that there are significant variations in the numbers of mantas reported pregnant over a year which supports the hypothesis that there is a mating season. Peaks in sightings of pregnant mantas would be likely to occur a month or two before the females delivered. The delivery month would be likely to coincide with the month of mating (from 12-13 months previous). These results suggest a mating season after July-August. The absence of any pregnant mantas being sighted in September weakens the results sets. Sighted manta were assessed for pregnancy up until 2011 but only 4 new pregnancies were identified since 2007 frustrating efforts to improve the results for analysis.

Case-histories of mantas assessed to be pregnant were be used to investigate periodicity. There were a total of 154 sighting of the 112 pregnant mantas reported in Table 10.2, with individual mantas being sighted pregnant 1-5 times. There were 337 other sightings of this group of mantas when not pregnant although 52 were seen only once. The most frequently re-sighted manta was seen not pregnant a further 29 times.

Using the state of pregnancy to predict likely conception and delivery months, the histories of all pregnant mantas were analysed and the predicted delivery month for every pregnancy reported in Table 10.3. Many of the deliveries were predicted to occur during or after the predicted mating season following the anniversary of likely conception but all observations were treated without bias to consideration of a mating season. A very heavily pregnant manta would be predicted to deliver in the following month, and one only just showing pregnancy would be expected to deliver in around 4 months (see examples in Figure 10.4). This method of prediction resulted in two pregnancies with a likely July or August delivery and a further four which fell outside

of the delivery period based on mating seasons (one in December 2003 and three in April 2003).

Year	Feb/Mar	Apr/May	Jul/Aug	Oct/Nov	Dec/Jan	**Total seen pregnant in year**	Total sightings all mantas	% pregnant vs. all sightings
2001				2		**2**	14	14.29
2002						**0**	68	0
2003	7	3			1	**11**	551	1.99
2004	4					**4**	473	0.85
2005	14			8		**22**	208	10.58
2006	5			1		**6**	402	1.49
2007	41		1	32		**74**	968	7.64
2008						**0**	136	0
2009	1		1			**2**	382	0.52
Total	**72**	**3**	**2**	**43**	**1**	**121**	3202	3.78

Table 10.3 Predicted parturition months for mantas observed pregnant October 2001 to December 2009.

Figure 10.4 Pregnant females 193 L163 'Dark Star' (left) and 235 L210 'Jaqui' (right) both seen during observations in late July/August 2007. The abdominal distension is obvious in both and suggests they are in last trimester but parturition is not imminent so an October birth might be anticipated.

According to the results in Table 10.3 there is a wide variation in the number of mantas reported pregnant each year (0-74) and wide variation in percentage of pregnancies with number of sightings (zero to ~14%). Data collection only commenced in October 2001, hence low count that year (and high percentage). However, pregnancy data were actively collected in the latter years of study; yet very few pregnant mantas were recorded in 2008 and 2009. The numbers of predicted births per month were analysed

using a Kruskal-Wallis one-way analysis of variance test with an adjustment for ties as there were so many zero results. There was a significant difference between the distributions between periods (H = 12.24, P = 0.016, DF= 4). The median number of predicted births for February-March was 4 and the median for all the other periods were zero indicating February-March as the period when most births were anticipated. A post-hoc analysis compared the February-March period with the other periods using a Mann-Whitney *U*-test. The distributions in the two groups for each pairing differed significantly for February-March with other periods (all at $P< 0.05$ level, adjusted for ties) except with October-November where $P= 0.231$. These results suggest there is a peak in predicted parturition in February-March (as well as October-November as already identified). As the period of birth would coincide with the mating period from approximately 12 months earlier these results suggest that there is mating season in February-March.

Periodicity of pregnancies

Eight mantas had multiple pregnancies between October 2001 and November 2009. Manta 243 L218 was sighted 35 times in this period and was observed in a highly pregnant state on 15.10.2005 and 2.11.2005. By the next sighting on 5.12.2005 parturition had occurred as she was no longer visibly pregnant. She continued to be observed throughout 2006 at cleaning stations on both sides of North Male atoll until 9.10.2006 when she was reported with no obvious mating scar. She was next sighted on 25.2.2007 with a new mating scar, and then reported on 28.5.2007 as visibly pregnant. She was observed pregnant throughout summer 2007 until early October. When sighted on 30.10.2007 she was no longer visibly pregnant so parturition must have occurred during October 2007. We may infer she mated soon after 9.10.2006 but was not seen after this mating until 25.2.07 (with the mating scar from October), and was then seen pregnant from late May onwards, at which point she would have been 7-8 months pregnant. Based on this cycle, the inference for the observed pregnancy in October/November 2005 is that she probably she mated in October 2004 too. This manta appears to have mated in the autumns of 2004 and 2006, and have delivered in November 2005 and October 2007, indicating an approximate 2 year mating cycle. The second pregnancy appears to have lasted 12 months confirming a 12 month pregnancy for mantas in the wild. She was seen with fresh mating scars in October 2007 and November 2009 but has not been sighted pregnant again, despite being seen several times each year.

16 S16 was observed heavily pregnant in October 2005, and again in February 2007 suggesting that births would have occurred very soon after the sightings. It is likely she delivered in October 2005 but did not conceive until the February-March 2006 mating season, suggesting ≈18 month cycle.

30 S30 was reported pregnant in October 2001, and again in July and August 2007; with the expected birth in October for both years when seen pregnant. She was seen in the interim (2002, 2004, 2005, 2006) but not reported pregnant, which suggests long gaps between pregnancies (>> 24 months). It is possible that she conceived in 2001 or 2002 and was simply not seen pregnant during those gestations but it is unlikely that she was pregnant from July 2004 until October 2006 unless she miscarried before the pregnancy was visible.

112 L79 was observed pregnant on 1.11.2005, with a likely delivery that same month: and again during October/November 2007, suggesting a November 2007 delivery. She was seen in July 2006 and was not pregnant. These dates suggest a 2 year cycle.

116 L83 was observed pregnant in February 2005, late October 2005 and late July 2007; indicating three cycles of pregnancy, not evenly separated. To be visibly pregnant for these dates she must have delivered in February 2005, and conceived almost immediately (12 month cycle) so as to be visibly pregnant by late October (she was seen in July 2005 and not reported pregnant which would be expected as the pregnancy would not show yet). She would have been expected to deliver in February/March 2006 but was not seen. The next conception must have been during the October mating season of 2006 for her to have been visibly pregnant by July 2007 (18 month cycle). She had fresh mating marks in November 2009, but has not been sighted pregnant since.

170 L139 was reported heavily pregnant on 28.12.2003 and again in October 2005. She probably gave birth by February 2004 and mated the following October (2004 deduced from the heavy state of pregnancy in October 2005), making a ≈ 18 month cycle.

280 L256 was reported pregnant October 2005 and October and November 2007 indicative of 2 year cycle.

313 L292 was reported pregnant December 2004, October 2005 and August to October 2007 indicating a 12 month cycle in 2004/2005 and then a 24 month cycle 2005/2007 as she was seen not pregnant between those dates. She could have delivered in late December 2004 and conceived immediately so as to be visibly pregnant the following October (2005).

10 cycles were reported. Two suggested a 12-13 months cycle (consecutive); three suggested ≈ 18 months cycle, apparently waiting until the next mating season to conceive; and four suggested an approximate 24 month cycle, apparently waiting a whole year to conceive after parturition. That none of these mantas have been reported pregnant since October 2007 and one frequently sighted manta apparently had a six year gap between pregnancies suggests that mantas may become pregnant much less frequently than every 1-2 years.

10.4.3 *Observations of courtship and other reproductive behaviours*

Two types of courtship are commonly observed: "shadowing**"** where the male rides on the dorsal surface of the females, and "chasing**"** as described by Yano et al (1999b).

Shadowing behaviour

Shadowing behaviour was commonly (during ~ 20% surveys) observed in the vicinity of cleaning stations and was reported throughout the year, not just during proposed mating seasons. A typical example which occurred at Lankan Reef on 26.7.2006 is described here. The female was already established on the main cleaning station and was being-cleaned when approached by male 490 L397. The male approached the female from above and behind until he appeared to be riding her and imitating her swimming movements (hence the term "shadowing"). The male used his cephalic fins to rub the female's dorsal surface. The full sequence is shown in Figure 10.5. The male's cephalic fins curled outwards and then towards each other alternately as he rubbed the female's dorsal surface, apparently to stimulate her to accept him for mating (see discussion). No mating occurred. After this sequence the male swam away and paid no further interest in the female. There was no obvious rejection behaviour from the female and it is unclear why the male separated so quickly. The female continued to be cleaned. Pairs of mantas have been observed to stay together (shadowing) for 10 to 60+ seconds. In some cases the female appeared to make attempts to swim away and

avoid the male or eject the male, behaviours which have been interpreted as rejection (Figures 10.6 and 10.7).

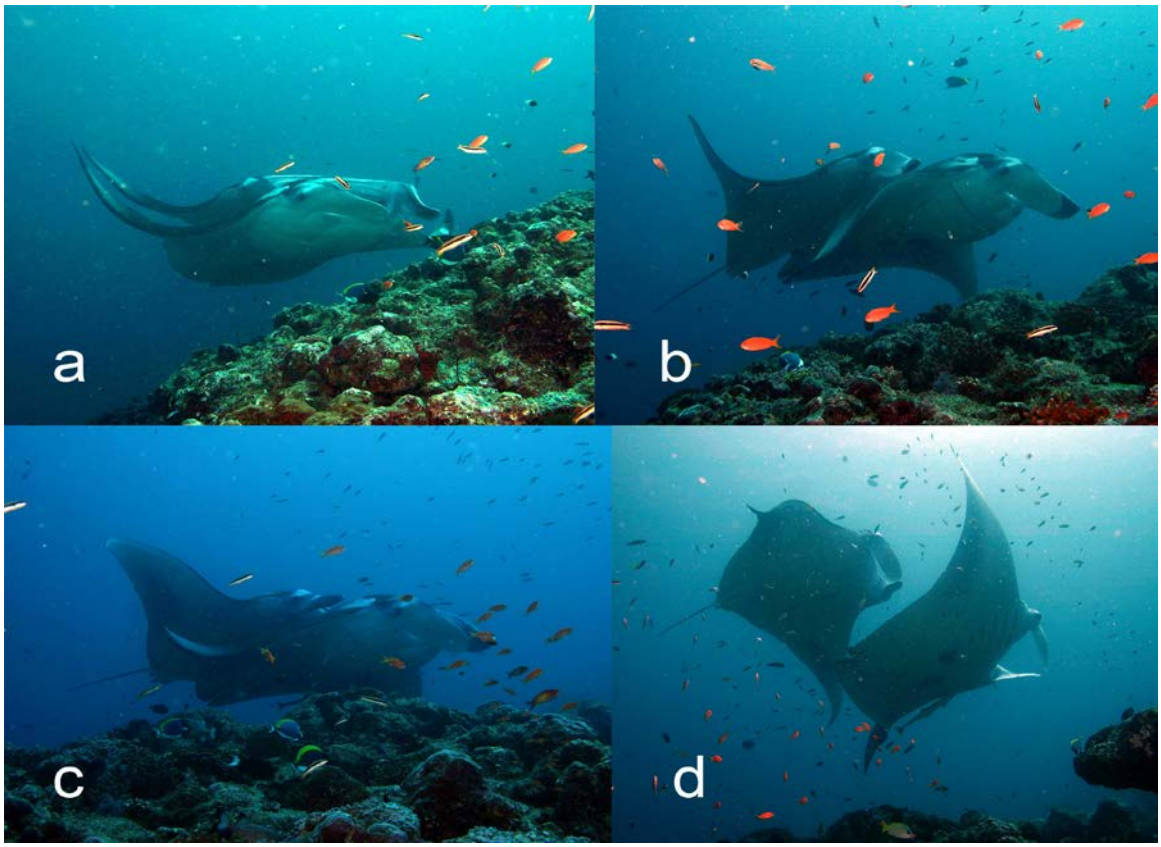


Figure 10.5 a) Male 490 L397 approached female 308 L287 from above and behind and started to ride her dorsal surface whilst imitating her swimming pattern, a behaviour termed "shadowing". **b)** The cephalic fins were unfurled and used to rub the dorsal surface of the female. This is likely to be a form of pre-copulatory releaser. **c)** The male's cephalic fins rolled to the furled position as he prepared to separate. **d)** The male separated from female despite lack of rejection by female

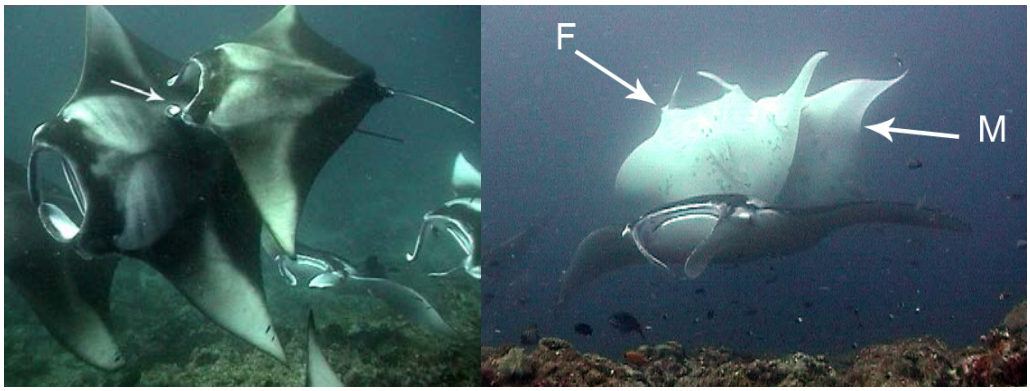


Figure 10.6 Left, a manta pair: the arrow indicates the cephalic fins of the male rubbing the female's dorsal surface. Right, female (arrowed F) rejected the male suitor (arrowed M) by performing an exaggerated backward roll.

Figure 10.6 shows a pair of mantas photographed during the autumn mating season in October. The female allowed the male to rub her for a short time and then rejected him by performing an exaggerated backwards roll to push the male off. There were several cases when pairs of mantas swam off together towards deeper water after shadowing, but whether copulation followed is unknown.

Courtship behaviour by adult males to pregnant females

During a full day observation at Boduhithi cleaning station on 9.1.2007, shadowing of females by males was observed throughout the day by various males to various females. Of 73 identified mantas, 37 were male (50.6%) and had a mean estimated DW of 2.87 m (SD ± 0.398, range 2.0-4.0 m) which is similar to the mean size of the all-mature male sample (2-sample *t*-test this sample vs. all adult male mean DW: $t = 0.85$, $P = 0.402$, DF = 4) so it may be assumed that the majority of these males were adult. Five pregnant females were present. There was no apparent pairing and multiple attempts by a number of single males to shadow different females were observed. Some females appeared agitated by the persistent males and one was seen to reject the male quite forcibly (see Figure 10.7). The males attempting shadowing were typically ≤3.0 m DW and whilst adult, their smaller size suggests that they may have been younger, and therefore inexperienced. Large (3.5-4.0 m) DW males were in the minority (10.8% of total males present) but did arrive, take part in cleaning activity in the presence of other mantas but none of the larger males were observed shadowing females. Some of the ≤3 m DW males had damaged claspers indicating previous mating experience.

A sequence of an attempt by a male to court a female is shown in Figure 10.7. The male, 1333 B109, was distinctive due to his missing right cephalic fin, and has an estimated DW of 2.5 m. The female was 1337 B113, a (estimated) 4 m DW, pregnant female. The male appeared very persistent and made repeated attempts to shadow the female who continually took evading action to prevent the male shadowing her. Her final motion was to swim into very shallow water so that the male was forced to drop behind her to avoid surfacing. The female then swam away with no further attention from the male. After this sequence both mantas re-joined the main group being cleaned and the male was not observed attempting to shadow any other female even though he was present for several hours and many suitable females were present.

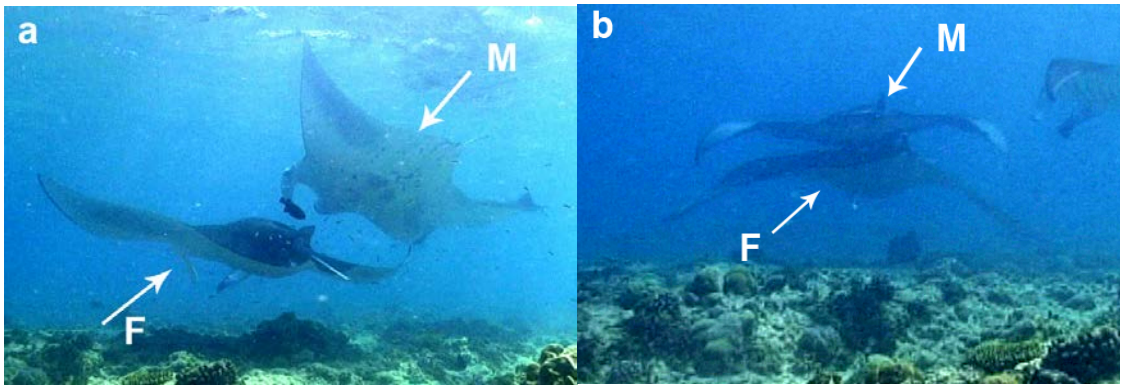
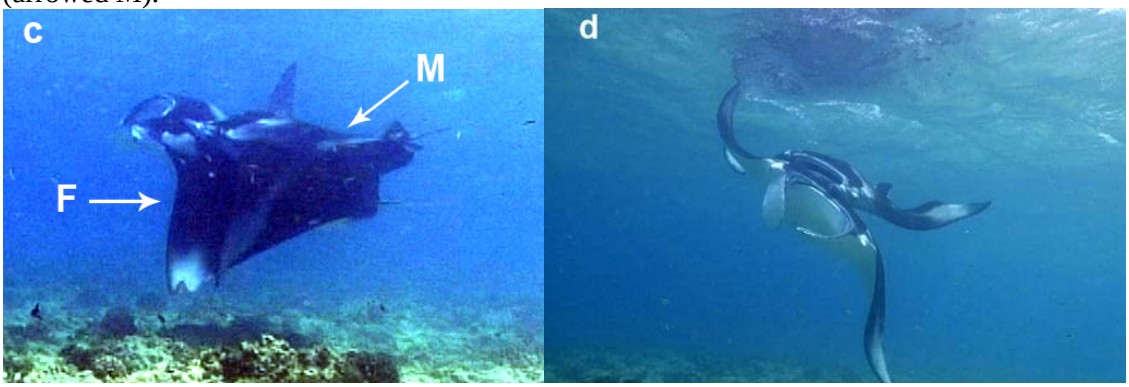
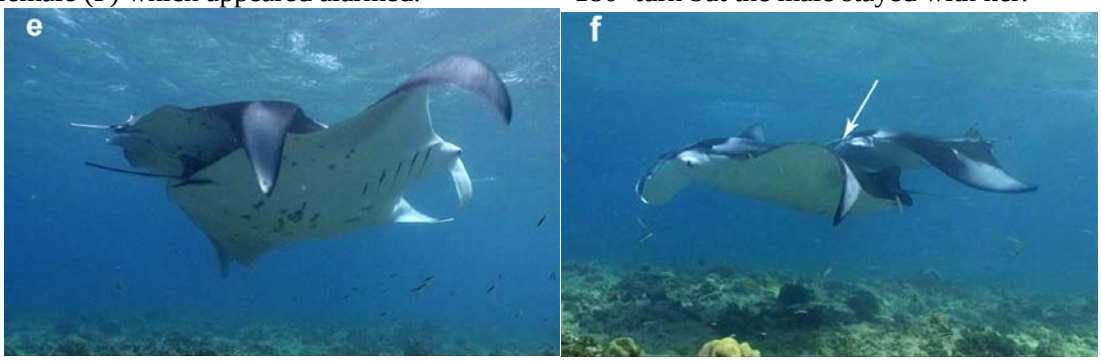


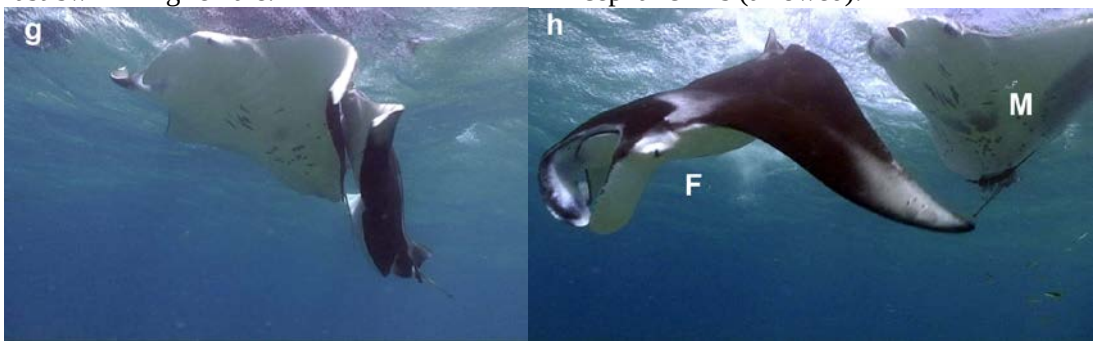
Figure 10.7 a. A pregnant female (arrowed F) was approached by a young adult male (arrowed M). b. The male (M) swam close over the female (F) to shadow her.



c. The male (M) continued to shadow the female (F) which appeared alarmed. d. The female (underneath) performed a fast 180º turn but the male stayed with her.



e. The male continued to stay close to the now fast-swimming female. f. The male attempted to rub female with his cephalic fins (arrowed).



g. The female (left) made a fast upwards swimming motion towards the water surface and the male was forced to fall back to avoid surfacing. h. The female (F) swam away alone. The male (M) made no further attempt to pursue the female.

Chasing behaviour

A female being chased by a single male, or a line of up to eight males (Figure 10.8), was commonly observed during the mating season periods, but was not observed outside these times. The line of males following the female is referred to as a 'train'(Yano et al. 1999b). The behaviour was not feeding behaviour as the cephalic fins were not held in the open feeding position. The train was always led by a single female, followed by males. The swimming pace appeared fast, but was not measured due to the events happening too quickly to be videoed. Observations at cleaning stations of normal cleaning behaviour were frequently interrupted by groups of chasing mantas, but the other mantas continued to be cleaned whilst the group of chasing mantas swam past (Figure 10.8).

Breaching events (where a manta jumped clear of the water) were also observed, either from underwater (rarely) or from the surface. Anecdotal observation suggested that breaching was most commonly observed during mating seasons but there were too few records for analysis.

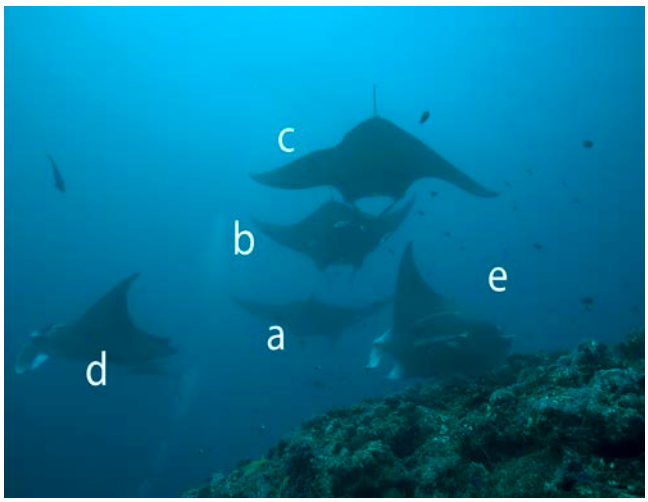


Figure 10.8 A photograph showing chasing behaviour at Lankan Reef cleaning station. Female (a) was chased by two males: (b) and (c). They swam over the cleaning station where two other mantas, (d) and (e) were being cleaned (note open cephalic fins for cleaning). (d) and (e) appeared un-distracted by the fast swim-past of the other three mantas

Mating Scars

Early observations of mantas noted that many large females had oval scars on the upper left wing tip. The scars were mainly white to grey but in some cases were black and were quite irregular (Figures 10.11, 10.12). Video of mantas mating showed the male securing the female by holding the left pectoral wing tip in its mouth; thus it was clear that these scars were caused by mating. This type of scarring had not been previously documented at the time of this study. There were no records of males with this type of scar and all (estimate >200 examples) observations in the Maldives were of the scar on

the left wing tip indicating a highly significant bias in selecting the left wing tip (χ^2 = 200, $P < 0.001$, DF = 1). There is no record of a fresh wound. All reports were of pigmentation variations referred to as scars.

Figure 10.9 Dorsal view of male manta showing white abrasions near dorsal fin and tail. These may have been caused by contact with the reef during copulation.

Figure 10.10 Dorsal view of mating scars on left wing tip inflicted by male from biting down to hold female in position during copulation and scratches (abrasions) from reef contact during mating act.

During a survey on 15.11.2008 at Lankan, of 14 females identified, 6 (42.9%) had obvious mating scars on the left wing tip. Mean estimated DW of females with scars and females without scars were compared and were found to not be significantly different (2-sample *t*-test, $t = 0.97$, $P = 0.351$). Of the six females with scars only one was seen pregnant the following year and none of the females without scars were reported pregnant. This suggests that mating does not necessarily lead to pregnancy in mantas.

Abrasion scars are frequently observed in the area around the dorsal fin on the posterior dorsal surface (Figures 10.9 and 10.10). They are reported on males and females. It is unknown precisely what caused the abrasions but it is proposed that they are caused during collision with the reef when copulation occurs in shallow water. This is the first report of this type of scar.

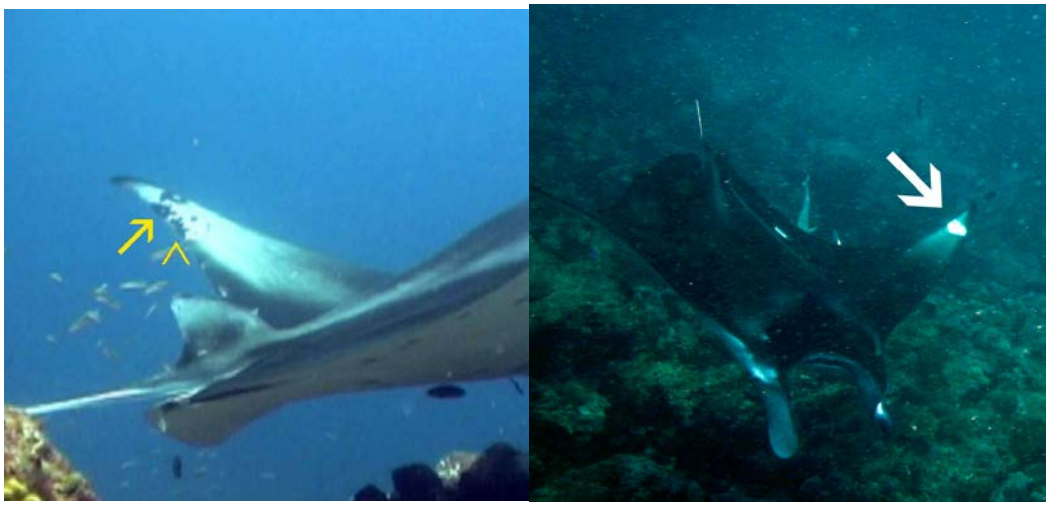


Figure 10.11 Photograph showing detail of mating scars on left dorsal wingtip of female mantas. In left example (yellow arrow), the scar pattern is grey-black and irregular. In right example (white arrow), the scar is a white oval on left wing tip

10.5 Discussion

10.5.1 *Gender mix and size*

The strong bias towards females in the population visiting the cleaning stations in the Maldives (63.9%) was less (Yates corrected $\chi^2 = 19.99$, DF = 1, $P < 0.001$) than seen in Mozambique, where Marshall and Bennett (2010b) found approximately 75% of the *Manta alfredi* population was female. They suggested this sexual segregation (Springer 1967; Klimley 1987; Sims 2003) might be due to reproductive strategies but no further explanation was given. The female biases observed in the Maldives and in Mozambique contrast to the population in Maui, Hawaii where only 46% were females (Deakos, 2010). The Hawaii result was significantly lower than the Maldives result (Yates corrected $\chi^2 = 32.99$, DF = 1, $P < 0.001$).

There was some variation in sex bias between sites in this study. The site with the highest proportion of females was Madivaru (2.7:1). This result was similar to the bias observed in the Mozambique population (Yates corrected $\chi^2 = 0.147$, DF= 1, $P = 0.702$). The lowest proportion of females was observed at Boduhithi (1.3:1) but this was still significantly higher than observed in the Hawaii population (Yates corrected $\chi^2 = 4.309$, DF = 1, $P = 0.038$).

Sexual segregation has been reported in other elasmobranch species e.g. scalloped hammerhead sharks *Sphyrna lewini* (Klimley 1987) or sandbar sharks *Carcharhinus*

plumbeus (Springer 1960), and it was suggested that the segregation was to maximise breeding potential. Klimley (1987) suggested that females congregate with the largest females in the centre of the school so that adult males may easily locate the largest (and thus assumed, most fertile) females. The sites where *M. alfredi* were reported in the Maldives were mainly cleaning stations. Feeding areas were much less frequently visited by observers and Chapter 8 (section 8.4.3) reported courtship-type behaviours whilst feeding but no courtship behaviour was observed when mantas were feeding. It is likely that mating occurs as an adjunct to being-cleaned, but we still know very little of the activities of mantas to make other conclusions. The rarity of sightings of actual mating suggests that none of the sites in this study were specific mating grounds.

Of the 198 males which could be assessed for maturity from photographs an unexpectedly high 97.5% were assessed as mature. This result is unlikely to be representative of the population as a whole; and may be caused by larger, more assertive males being less frightened by the presence of divers and thus be more likely to be photographed than smaller mantas. Alternately, it may be because juvenile and sub-adult males do not visit cleaning stations very frequently (or at least, not the cleaning stations studied). It appears that the mean estimated size of these mature males and the known mature females from the Maldives are smaller than those reported mature from Mozambique where males *c*. 2.8m DW were still immature and the majority of pregnant females were >4.0 m DW (range 3.9-5.3 m DW). In the Maldives, no *M. alfredi* were estimated or measured >4.5 m DW so Maldives mantas may be of a smaller size generally. Insufficient data were available from Marshall and Bennett (2010b) to make statistical comparison.

10.5.2 *Periodicity of mating activity and pregnancy*

The Rayleigh tests indicated there were significantly non-uniform distributions of the percentage of female mantas sighted pregnant (amongst the peer group of mantas ever-known pregnant) throughout the year. The results suggested that a peak in mating activity or a "mating season" would occur 2-3 months after July/August each year as that was indicated as the time of year when most pregnant mantas were sighted. This would concur with the hypothesis that there is an October-November mating season. The results of the investigation to predict parturition based on assessment of the state of pregnancy indicated a peak in parturition during the February-March periods between the years of 2001 and 2009 providing evidence of a second mating season. Taken

together, these results provide evidence to support the hypothesis that there are two mating seasons, around October-November and February-March. That several mantas were expected to give birth outside the mating seasons suggests that mating and conception may occur at any time.

In Mozambique, Marshall and Bennett (2010) reported a single mating season during the Austral summer from October to January with mating bouts being concentrated over periods of a few days occurring several times during the season, but mating activity being observed exclusively during this period. This is different to the results from the Maldives where there appeared to be two shorter mating seasons, coinciding with the latter stages of the two seasonal monsoons. During the period at the end of the monsoons larger numbers of mantas were observed at cleaning stations (Table 10.2) and mass-feeding events were sometimes observed (Chapter 8). The relationship of high productivity and mating activity is discussed in section 10.5.4.

The female at Okinawa Aquarium conceived immediately after parturition (Matsumoto and Uchida 2008; Uchida et al. 2008). The evidence from pregnancies reported in two mantas from this study and five from the Mozambique study confirm that mantas in the wild may have consecutive pregnancies. All seven appear to have conceived very soon after parturition, so as to be pregnant during the following year. Additionally, adult male mantas were observed courting heavily pregnant females (Figure 10.7). It is likely that mating and conception follows parturition in the wild too. Elasmobranchs which may conceive immediately after parturition are termed continuous breeders as follicular development is continuous, even during pregnancy. This occurs in many viviparous sharks (Springer 1960; Wass 1973; Pratt 1979; Koob and Callard 1999) and rays (Mellinger 1974). Of the other eight mantas with multiple pregnancies recorded in the Maldives, four indicated a ~24 month cycle. In practice this would consist of a mating resulting in conception, followed by a gestation of 12-13 months until birth, followed by a break of approximately 11-12 months with the next conception occurring around the second anniversary of the previous conception. A 24 month cycle was also more commonly reported in the Mozambique study. There were three examples of ~18 month cycles in the Maldives suggesting the second conception occurred during the following (hypothesised) mating season, i.e. a parturition occurring in February/March with the next conception occurring during the October mating season which would result in a 20 month cycle or a parturition occurring in October followed by conception in February

resulting in a 16 month cycle. The results indicate that, more commonly, mantas in the Maldives do not conceive soon after parturition. Instead, conception occurs during the following mating season or even the mating season around the anniversary of parturition. It is unknown whether such mantas mate, but fail to conceive, or whether the females avoid mating until a later mating period. I speculate that it may be beneficial for the female to be pregnant during alternate years to allow the female to fully recover from the pregnancy and continue to grow in size and strength. A gap might increase the likelihood of a successful next pregnancy as producing large offspring is energetically costly and the female may need to recuperate energy stores after the pregnancy (Carrier et al. 1994). In addition, observations over an eight year period indicate the frequency of pregnancy to be less than bi-annual, with very few pregnancies observed since 2007. I can offer no explanation for the large number observed in both February-March and October 2007, nor for the low number of pregnant mantas observed after 2007 since surveys were carried out during the hypothesised mating seasons of all years and throughout each of the years. Low birth rate combined with a single pup (Coles 1916; Beebe and Tee-Van 1941; Bigelow and Schroeder 1953; Marshall et al. 2008) produced from each pregnancy indicates a very low fecundity. It appears that *M. alfredi* has one of the lowest known fecundities, even less than the protected sand tiger shark *Carcharias taurus* Rafinesque, 1810 (IUCN 2008). Elasmobranch fisheries are generally unsustainable (Musick 1999; Dulvy et al. 2008): manta populations would be less able that other elasmobranch populations to recover from targeted fisheries.

10.5.3 *Observations of courtship and other reproductive behaviours*

Chasing and copulation behaviour of *Manta birostris* was previously described by Yano et al. (1999), and more recently of *M. alfredi* by Marshall and Bennett (2010b). Courtship behaviour by a number of males towards a single female was reported by Nolan (1996) in Ocean Realm magazine. The behaviour involved males riding the dorsal surface of the female until rejected by the female which was described by Nolan as mating behaviour. Based on observations described in section 10.4.3, he had recorded “shadowing” courtship behaviour.

It appears that shadowing courtship behaviour is performed throughout the year and is observed without mating necessarily taking place. In contrast, chasing is only observed during the mating seasons in the Maldives. It appears that either form of courtship may lead to mating. Shadowing is more commonly observed, usually does not lead to

copulation, and often results in dismissal by the female involved. Pregnant mantas are commonly shadowed and this may be because males are generally attracted to large females. Larger female scalloped hammerhead sharks *Sphyrna lewini* have greater reproductive capability and produce larger litters than smaller sharks and male sharks are known to go to the middle of the school to locate the largest females (Klimley 1987). An alternative hypothesis for males to be attracted to pregnant females as proposed by Carrier et al. (1994), is that mating follows parturition, which may act as a stimulus or readiness cue for elasmobranchs. The stimulation behaviour observed in shadowing may be a form of pair-bonding and the acceptance by the female suggests that female mantas may select their male partners. In cases where the female appeared to accept the male, the two mantas swam off together towards deeper water, presumably to mate. Figure 10.5 shows an example where both mantas separated without any sign of dismissal by the female, who did not appear pregnant. It is unknown how or why the female selects (or dismisses) a mate from shadowing and stimulation behaviour. An alternate hypothesis to the female selecting a male is that the female is already pregnant (but not visibly so) and therefore unable to conceive so does not respond to stimulation. Non-biting stimulation by males to females was also observed in the courtship rituals of giant guitarfish *Rhynchobatus djiddensis* (Forsskål, 1775). Males swam past the females approaching from either posterior dorsal side. As the male passed, he collided flank to flank in a purposeful manner. In this species the males are approximately half the length of the female and the bumping appeared gentle, perhaps stimulating the female, and not violent or aggressive (pers. obs.).

In the manta shadowing sequence shown in Figure 10.7, the female behaviour was non-accepting yet the male persisted. It is unknown whether the male selected the female specifically because she was heavily pregnant (and thus would potentially be ready to mate again soon) or was just attracted by her large size. Both animals involved demonstrated great agility and manoeuvrability. As the female was pregnant, no mating could have followed immediately so the actions could be for practice or pair bonding. Alternately the female manta dismissed the male specifically because she was pregnant, concurring with the alternate hypothesis above. It will not be possible to confirm that female mantas which dismiss potential suitors do so because they are pregnant without taking blood samples from females which do not appear to be pregnant, which will be problematic.

Chasing behaviour was commonly observed during the main mating periods of February/March and October/November. The swim speeds during this activity were higher than average (Yano et al. 1999b), but were not measured in this study as observed sequences were too short to analyse or visibility interfered with analysis. The swimming speeds appeared to be at least as fast as when feeding (Chapter 8). It may be perceived that the mating following chasing is forced on the female. However, as females may make selection following shadowing behaviour (the female may dismiss the male who then swims away, or accept the male and they swim into deeper water to mate), the female may also be selecting the male most able to chase her and demonstrate superior manoeuvrability. Thus, the female selects the male(s) to mate with her after chasing behaviour too. In support of this hypothesis is that the females being chased are larger than the pursuing males; and the size advantage should give them more speed and power during chase. Additionally, no aggression has ever been observed by a group of males towards a female. This is in contrast to group mating by white tip reef sharks *Triaenodon obesus* (Tricas and Feuvre 1985; Whitney et al. 2004) or nurse sharks *Ginglymostoma cirratum* (Carrier et al. 1994), in which a number of male sharks aggressively restrained a female by biting on her gill slits and took turns to mate. Selection of a mate by the female from a group of pursuing males was recently described for humpback whales *Megaptera novaeangliae* in Hawaii (Herman et al. 2007). A hypothesis for why chasing only occurs during the peak mating season is that there may be many males seeking females with which to mate and chasing allows only the fittest males to be allowed to mate. However, there was no evidence from data on the male sightings that males were more common during mating periods.

It is known that female mantas may mate with several males consecutively (Yano et al. 1999b), yet observations from this study have shown that mating does not necessarily lead to conception (or early miscarriage may be relatively common).

In this study the first of five key mating steps reported in Yano et al. (1999b) and Marshall and Bennett (2010b) i.e. chasing, did not occur in the majority of courtships observed. Instead, chasing was replaced by shadowing and stimulation of the female using the male's cephalic wing tips on the female's dorsal surface. It was proposed here and in Marshall and Bennett (2010b) that the female may select a mate. An alternate hypothesis is that outright rejection is only made by pregnant females who are unable to respond to stimulation and do not excrete the olfaction cues necessary to initiate chase

behaviour. This hypothesis would require that most adult females are pregnant for most of the time (based on the rarity of which acceptance by a female, leading to mating is observed), and the rarity of mantas sighted in the latter stages of pregnancy does not support this.

Healing of elasmobranch tissue in less than 12 months from scars and missing tissue was reported by Mourier et al. (2007) for individually identified sicklefin lemon sharks *Negaprion acutidens*. Wounds and scars on the dorsal side of the left wing-tip of females were caused by mating activity when the male grips the female with his mouth on the left wing tip. Female mantas were frequently observed with such mating scars which appeared to last for several months after mating. Database records for individual mantas indicated wounds healing completely over a 4-12 month period during which the scar disappeared and new wounds/ scars were reported. These observations for mantas indicate a similar healing rate to the lemon sharks. The wing tip mating scars were consistently observed on the left side of the female indicating strong lateralization in the approach by male *M. alfredi* before copulation. This may be another example of stereotyped etiquette by mantas generally, as a left approach was also observed in males of *M. birostris* (Yano et al. 1999b). Biting has been proposed as a pre-copulatory releaser (Springer 1960), but was only involved in the latter stages of copulation in mantas and may merely be used by males to manoeuvre into, and maintain position for mating (Kaijura et al. 2000). Biting is not employed as releaser by other myliobatoids e.g. spotted eagle rays *Aetobatus narinari* and bat rays *Myliobatis californica* (Tricas 1980).

10.5.4 *Mating seasons in the Maldives and food abundance*

The timing of the two proposed mating seasons may be linked to food abundance in the Maldives, but this has yet to be demonstrated. The proposed mating seasons coincide with the periods towards the ends of the two seasonal monsoons when conditions (wind direction and water movement) are consistent, so feeding opportunities for mantas are likely to be optimal on the leeside of atolls (Anderson et al. 2011). It is during these periods that large aggregations of mantas are commonly observed at inshore sites throughout the Maldives[2].

[2] The highest numbers of female manta sightings were recorded during February, March and October (Table 10.2). The high result obtained for July was due to the disproportionately high number of surveys conducted at Lankan during July for the population and cleaning studies.

A link between food abundance and mating activity may occur in basking sharks *Cetorhinus maximus* (Sims et al. 2000) where individuals aggregated to forage in rich prey patches before initiating pre-courtship. Also, Simpfendorfer and Milward (1993) showed a link between prey abundance and the number of juveniles (of at least eight species of sharks of the families Carcharhinidae and Sphyrnidae) found in sheltered lagoons. The abundance of juveniles may have been caused by adult females giving birth in areas with high productivity to give offspring the best chance of success. High food abundance for manta offspring would be an important attractor of large pregnant females to particular areas as both adults and offspring would benefit. All the main survey sites have large sheltered lagoons nearby, which might make suitable protected environments for the first few years of life of a manta. Juvenile mantas have been sighted (but not identified and thus the database is deficient in juvenile mantas) in many of the lagoons next to resorts or local islands where boats anchor including Maayafushi, Rasfari, Ran Faru and even inside the man-made lagoon of the artificial island of Hulhumale. Juveniles of other elasmobranch species are known to aggregate in nursery areas such as lagoons, mangroves and sheltered bays (Castro 1993; Simpfendorfer and Milward 1993; Heupel and Simpfendorfer 2005). It was proposed that the segregation of juveniles protects the young from predation by other sharks of the same species (where applicable) or other species, and reduces competition for food with adults (Last and Stevens 1994; Guttridge et al. 2009a). A birth of a manta in the wild has yet to be recorded.

10.6 Next steps

The sex ratios reported were for cleaning stations. As knowledge on other locations where mantas are sighted and identified grows it should be possible to have large enough data sets from feeding sites and even sheltered lagoons from which to make useful analyses. It is expected that the sex ratio in the sheltered lagoons would be closer to 1:1 as that is where young juveniles tend to be observed and this ratio is what would be expected from sexual reproduction, but sexual segregation may still occur.

This population of wild mantas appears to have infrequent full-term pregnancies and should be monitored over the long term. Very few pregnant mantas were observed between 2008 and 2011 (only the results to 2009 were reported in the study) but I can offer no explanation as high numbers of mantas were reported each year and environmental conditions seemed stable, suggesting their food source was not adversely

affected. My intention is to continue this study for a further 10 years to improve this dataset. If female *M. alfredi* in other locations have similar low birth rates (as was suggested by Marshall and Bennett 2010b in Mozambique), then this species requires protection or it will be removed from areas where it is actively fished.

Chapter 11. General discussion and conclusions

11.1 Introduction

Knowledge of *Manta alfredi*, the most commonly sighted manta reported in the Maldives, has grown considerably as a consequence of this, and other studies conducted at the same time. The amount of published material on genus *Manta* has also grown considerably. At the commencement of this study there were a handful of scientific papers, fish guide entries of ~200 words and historical records, now there are 45 peer-reviewed articles in scientific journals and scientific conference abstracts. The most important change is that there are at least two recognized species of manta, with evidence supporting the third species in review (A. Marshall, pers. comm.). Evidence from this study involving the differences in ventral markings between the species supports the hypothesis that there is more than one species. Many of the discoveries from this study including range of sizes, size at maturity, migration behaviours and population estimates provide further support to there being several species of *Manta.* Important discoveries on manta feeding, being-cleaned and social behaviours can be used in tourism management plans where knowledge on the behaviour of mantas is essential so that appropriate actions are taken to minimize manta habitat damage and harm from tourist interactions.

11.2 Individual identification from markings patterns

1940 different *Manta alfredi* have been identified, so far, using the method described in Chapter 4 and reported in Kitchen-Wheeler (2010). The ventral markings on each *M. alfredi* appear to be unique. As long as a good record (a clear photograph or video) of the ventral markings and other criteria (sex, tail length) is obtained, all mantas recorded have been individually identified. An all-black or all-white animal could not be identified but so far none have been reported. Using the ventral markings for individual identification has been adopted as the standard method of identification by other researchers in the field (Couturier et al. 2011; Deakos et al. 2011; Marshall et al. 2011) (F. MacGregor, M. De Rosemount, G. Stevens, pers. comms.). The markings patterns of *M. birostris* are more complicated than those of *M. alfredi* and there were fewer samples from which to make conclusions (8 in the Maldives plus approximately 200 from datasets from other locations) but these also appear to be unique to the individual and have been used to identify individuals in local populations (Luiz et al. 2009; Kashiwagi et al. 2011) (K. Kumli, M. Harding, pers. comms.).

Mantas are born with a full set of ventral markings (Marshall et al. 2008; Matsumoto and Uchida 2008) and markings did not appear to change significantly over the period of this study with examples suggesting no change over 7 years (Figure 4.12) and anecdotal evidence suggesting insignificant change over 25 years. These results are important as they suggest that individual mantas of both species may be recognised throughout their lifetimes by their ventral markings patterns. Thus, this method can be used to identify individuals during migration, behavioural, environmental or population estimate studies spanning several years, and possibly, decades.

11.3 Locations where manta rays are sighted

In this study the majority of mantas were reported from cleaning stations and the remainder from inshore feeding areas. Most of the cleaning stations were located on the atoll outer reefs or in channels just inside the atoll (Figure 5.2). Some cleaning stations were considered reliable for sightings of mantas; for example mantas were reported during ~85% of seasonal surveys at Lankan, however the numbers of mantas reported varied significantly from one survey to the next (range 0-34, mean =7.09±0.511 SD, median=5 at Lankan). Overall, the modal number of mantas reported during a survey was 1. The mean time spent by a manta at Lankan was 35 minutes, but some individuals spent several hours in a single day there. The population study (Chapter 6 and reported in Kitchen-Wheeler et al. (2012)) suggested site and atoll populations of hundreds of mantas. It is apparent from the low number of mantas sighted together at cleaning stations and during feeding events that individual mantas visit the known cleaning areas and feeding areas relatively infrequently, and that we still do not know where they are for the majority of the time. There were a few surveys when large numbers of mantas were reported: Table Thila when it was estimated that around 100 mantas were present (Table 7.7); Koshibee Kanduoli (section 8.4.3) where it was estimated that around 150 mantas were feeding together and at Hanifaru where up to 50 mantas feeding together was observed once or twice a month (Table 6.1). These latter observations suggest that the majority of mantas from a local population may converge to take part in mass cleaning or mass feeding activities, but these events are relatively uncommon.

The main predictor for the locations where mantas may be sighted is season. Results from Chapter 5 showed that individual mantas migrate from east to west sides of Maldives archipelago with the change in prevailing monsoon so as to be found at sites on the leeward sides of the atolls. In particular, they would be expected to be absent

from sites on the outer reefs of atolls which became windward. It was shown that in North Male atoll individual mantas migrated from east sites (Lankan, Sunlight thila, Kani and Aquarium and Fairytale reef) where they were sighted during the south-west monsoon, to a western site (Boduhithi) during the north-east monsoon. Anderson et al. (2011) showed a correlation of the presence of mantas and Chlorophyll-a levels, suggesting that mantas were found where high levels of plankton were located. It appears that the seasonal migration in the Maldives is likely to be caused by mantas following their food source. Movements by mantas caused by variations in food availability were also proposed in other locations including eastern Australia (Couturier et al. 2011), Komodo (Dewar et al. 2008) and south-eastern Brazil (Luiz et al. 2009). In all cases the mantas were observed at, or moving to, areas of relative plankton abundance. Reports of seasonality in other large planktivorous elasmobranchs such as basking sharks, whale sharks and mobula rays were proposed to be related to temporal variability in the abundance of their zooplankton prey (Notabartolo-Di-Sciara and Hillyer 1989; Taylor 1996; Sims and Quayle 1998; Wilson et al. 2001). The seasonality reported in the Maldives is also likely to be due to zooplankton abundance but a direct correlation has yet to be established.

Whilst seasonal migrations might be explained by changes in their food source, a suitable hypothesis for the north-south movements between sites on the same side of an atoll or between atolls is less forthcoming. The level of inter-atoll movement was low with only ~1% of mantas reported in different atolls. There is no clear explanation for this small number of mantas travelling hundreds of kilometres between different cleaning stations. Mantas are large pelagic free-ranging fish with daily movements of 40 km between feeding and cleaning station sites recorded (Clark 2008; Dewar et al. 2008). A one-way travel of 80 km between cleaning stations in North Male and Baa atolls (section 5.4.3) could realistically be performed over one or two days, so this type of movement should be expected. Reported in this study and by Kashiwagi et al. (2010) were individual *M. alfredi* making one-way, long distance (~300 km) movements requiring the traverse of deep water (>600 m deep). This type of movement is similar to that performed by *M. birostris* (Rubin et al. 2008; Marshall et al. 2009). That both species are seen at shallow reefs during the day visiting cleaning stations and feeding areas, both travel offshore to visit feeding areas at night and both are capable of movements of several hundred kilometres suggests that the movements of both species are more similar than comparisons in earlier reports on the two species might have

suggested (Marshall et al. 2009; Couturier et al. 2011; Deakos et al. 2011; Marshall et al. 2011). The two species are sympatric throughout much of their range (Marshall et al. 2009; Kashiwagi et al. 2011). The main difference is that *M. birostris* is more seasonal in its visitation pattern to areas where it is reported (Rubin et al. 2008; Luiz et al. 2009; Freedman and Shouraseni 2012) (M. Harding pers. comm.), and this may be due to the species inhabiting areas prone to broader variations in water temperatures and consequent variations in prey availability. Presently we do not know where individual *M. birostris* go when they are absent from the areas where they are seasonally recorded although Rubin et al. (2008) recorded one *M. birostris* at locations 613 km apart (the Revillagigedos islands to the Sea of Cortez) which is further than the known distance travelled by any *M. alfredi* in one direction.

11.4 Behaviours of *Manta alfredi*

Mantas were most commonly encountered at cleaning stations in the Maldives. This study suggests that individual *M. alfredi* visited cleaning stations daily. The time spent at the cleaning station ranged from a few minutes to several hours with a mean time of around 35 minutes. The results from the time spent cleaning study were similar to those reported by O'Shea et al. (2010) in eastern Australia so may be representative of typical time spent at a cleaning station in a day by this species.

It appeared that cleaning was not the only activity performed by mantas in the vicinity of the cleaning stations as being-cleaned activity was frequently interrupted by a courtship behaviour termed “shadowing”, by males towards females. Additionally agonistic displays between pairs of mantas termed “head-offs” were frequently reported. It appears that time at cleaning stations is also spent in social interactions.

The five main cleaners of mantas in the Maldives were *Thalassoma amblycephalum, T. lunare, Labroides dimidiatus, L. bicolor* and *Bodianus diana*. *T. amblycephalum* had not been reported as a cleaner elsewhere, yet appears to be the most important (by number) in the Maldives. Large groups of juvenile *T. amblycephalum* and *T. lunare* were reported at the majority of cleaning stations yet juveniles are not mentioned as cleaners in references (Cheney 2008; Oliver et al. 2011) (www.fishbase.org species profiles access date 29.5.2012). The number of individual cleaner fish observed with a manta (and assumed to be cleaning) varied from 1 to 117. There was no correlation of numbers of mantas being cleaned with the number of cleaner fish, nor size of the area of

cleaning stations. These results concur with the hypothesis of Bshary and Schaeffer (2002) which suggested that there may be “hotspots” of preferred cleaners, and might explain why the one block at Lankan was consistently used by mantas whilst the other blocks (which contained similar numbers and species of cleaners) were ignored.

This knowledge on the behaviour of mantas at cleaning stations is important in protecting the income from tourism based on diving and snorkelling with mantas. The majority of sites visited by scuba divers to see mantas are cleaning stations. It is imperative that divers should avoid damaging the reef at these locations as it is home to the cleaner fish. With a damaged environment the cleaners cannot thrive and the cleaning station would be lost. Whilst natural events may cause damage to the reefs e.g. coral bleaching or storm damage, these are unavoidable. Damage from unregulated tourism or construction works is not. All of the well-known “manta points” should be designated as Marine Protected Areas and regulations for diving practices in these areas enforced (Ministry of Tourism 2003).

Results from the investigation of associations between the 84 most commonly seen mantas in North Male atoll suggested that they did not form close bonds with a small number of mantas but instead had loose associations with the majority of other mantas within the sample group. Mantas with the lowest mean index of association (IA, i.e. the weakest links with other mantas) were seen significantly more frequently than mantas in the high mean IA group. This suggested that mean IA might be used as a measure of sociability and mantas with a low mean IA might have a bold personality trait as they were most frequently seen at the cleaning stations and more likely to be sighted on their own or in smaller groups (sections 9.4.1 and 9.5.1). Differences in personality might explain the high ratio of females to males of 2.6:1 obtained at Madivaru. Madivaru is the most valuable dive site in the Maldives by revenue (Anderson et al. 2010) with hundreds of divers visiting on a daily basis throughout the NE monsoon. The regular presence of divers is likely to deter all but the most bold of mantas and this hypothesis is supported by the high proportion of females seen there and the declining numbers of mantas reported per survey at Madivaru between 2002-2009 (the regression between number of mantas seen per survey and year had a weak negative correlation: $F= 3.37$, $P= 0.071$, $DF=1$). The mantas which visit Madivaru are likely to have access to many cleaning stations along the western side of Ari atoll and would use them in preference to

Madivaru, so only income from manta tourism will suffer in the long term if nothing is done to manage numbers of tourist scuba divers at this, and other popular sites.

Snorkelling with mantas is the other major generator of income from mantas in the Maldives. This activity primarily occurs when mantas are feeding in shallow water. This study showed that feeding is a dynamic activity with mantas swimming nearly twice as fast as when being cleaned (wing beat speed was ~30 bpm when feeding vs. ~16 bpm when being-cleaned, section 8.4.4). Whilst feeding, mantas employed specific movements which likely optimised their swimming through areas of concentrated plankton. These movements included horizontal feeding in line or tornado formations, somersaulting and barrel rolling, vertical feeding and hunting-feeding. Feeding appears to be a group activity and it was proposed that a back-ward somersault followed by a vertical descent might be a form of intraspecific communication as when the behaviour was observed performed by one manta, a series of other mantas imitated it. It was proposed in Chapter 8 that a manta might be using the bright "flash" produced when it somersaulted to communicate to other mantas where food was located. This behaviour, along with the organized lines and stacks of mantas observed during horizontal feeding strategies, suggest that mantas behave co-operatively and not competitively whilst feeding.

11.5 Ecology and economics

The expected longevity of *Manta alfredi* is still unknown. Based on the maximum known age of 36 years (section 2.6) the predicted age of sexual maturity was 13. The first successful birth was observed from a female manta held in captivity and estimated to be 13 years old at the time of mating (Uchida et al. 2008). It is likely that the maximum longevity of this species is much greater than the known 36 years and the 13 years age of maturity is a minimum estimate. The vulnerability of this species discussed in Chapter 10 due to low birth rate, small litters and late sexual maturity cannot be stated strongly enough.

Both *M. alfredi* and *M. birostris* are economically important in most of the areas where they have been studied (Australia, Mexico, Ecuador, Mozambique, Hawaii, French Polynesia, Maldives, the Caribbean) and support valuable diving or snorkeling with mantas tourism activities (Anderson et al. 2010). Unregulated, these activities may drive the mantas from the more accessible locations, but generally do not harm the mantas. If

the activities were regulated to moderate the number of people and best practices for maintaining pristine environments for the mantas were implemented; then manta tourism might be sustainable, and could be developed to become even more valuable to local economies. It is important that activities with mantas are managed so as to minimize effect on the mantas' natural behaviour. Manta fisheries are unsustainable but manta tourism need not be.

When I commenced my data collection in 2001 I wanted to know how many different mantas I was encountering and to understand what I was seeing at the cleaning stations. I also wanted to be able to predict the best times to find mantas. Whilst it was expected that each atoll supported a population of several hundred different animals, it was surprising to discover that the mantas encountered appear to be split into ones which were seen very regularly and those which were sighted only once. The regularly sighted mantas (which are in the minority) have been named and have been re-sighted over many years performing mating rituals, bearing mating scars and becoming pregnant. They swim through our exhaled bubble streams and pose for our photographs so have become easily recognizable and are locally famous. Individually they are very valuable to the Maldives economy as they account for the majority of mantas reported at the main tourist diving and snorkeling sites.

Results from this study suggest that individual mantas are likely to be cleaned daily, but the reason we saw mantas in small numbers and so infrequently was because we spent, proportionally, very little time in survey and could only survey one cleaning station at a time. By way of predicting the presence of mantas, this study confirmed that they would likely be located at cleaning stations and feeding sites on the leeside of the atolls as anticipated. However, tide state appeared to have little effect on number of mantas present and at Lankan (the most investigated site) more mantas were sighted during the early afternoon. Both these latter results were unexpected. Anecdotal evidence suggests that current may be more useful in predicting good times to see mantas as they avoided periods of high current at some sites but this relationship may be site specific. The effect of current (with site) will be the subject of future investigation.

Knowledge on the behaviour and ecology of this species has grown considerably as a consequence of this study, but there is still relatively little knowledge on their diel activities. There is a recent report by Graham et al. (2012) of mantas reported as *Manta*

birostris from the Yucatan Peninsula, Mexico. These are likely to be *M. giorna* based on the locality and the likelihood that the species will be separated in the near future (Marshall et al. 2009) (A. Marshall, pers. comm.). The study described satellite tagged mantas in a foraging state for 97.7% of the locations received from the Argos tracking system. Although individuals travelled up to 1,151 km (over a period of weeks), they moved to locations a maximum of 116 km from the point of attachment, and remained further than 20 km offshore at 92% of locations. From its restricted distribution, *M. giorna* appears to replace *M. alfredi* species in the Caribbean and North West Atlantic; behaving in a similar manner by remaining in a smaller area year-round and migrating shorter distances between locations where reported. Based on the low numbers of *M. alfredi* sighted inshore at feeding or cleaning areas in the Maldives compared to known local populations, it is likely that they also spend the majority of their time offshore, foraging over deeper waters. It would be useful to satellite tag some individual *M. alfredi*, particularly in locations where they are known to travel greater distances (Maldives, Eastern Australia, Yaenema islands Japan) to investigate this hypothesis.

It is expected that within the next 5 years that the amount of information about mantas rays will re-double due to the economic importance from tourism of these species.

Appendix A. Published papers with content from this study

Visual identification of individual manta ray (*Manta alfredi*) in the Maldives Islands, Western Indian Ocean

Anne-Marie Kitchen-Wheeler

Marine Biology Research, 2010; 6: 351-363

Extent and economic value of manta ray watching in the Maldives

R. Charles Anderson, M. Shiham Adam, Anne-Marie Kitchen-Wheeler and Guy Stevens

Tourism in Marine Environments, 2010; 7: 15-27

Population estimates of Alfred mantas (*Manta alfredi*) in central Maldives atolls: North Male, Ari and Baa

Anne-Marie Kitchen-Wheeler, Csilla Ari and Alasdair J. Edwards

Environmental Biology of Fishes, 2012; 93: 557-575

Marine Biology Research
2010, 1–13, iFirst article

ORIGINAL ARTICLE

Visual identification of individual manta ray (*Manta alfredi*) in the Maldives Islands, Western Indian Ocean

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Abstract
Despite the worldwide distribution of manta ray (*Manta alfredi*) in tropical and subtropical regions, there is very little published information on their biology or ecology. Knowledge of the abundance of mantas and their migration patterns is essential for conservation, and the first stage in any study is a method to identify individuals. The purpose of this paper is to present a method of visual identification of individuals of manta which can be used at any site where mantas are regularly observed. In mantas, each individual has a characteristic pattern of dark markings on the ventral side. The most important individual identifying marks are patterns of ovals in the area between the gill slits, supported by distinctive patterns of mottles and spots in the lower abdominal area (posterior to the gill slits) and areas of dark pigmentation around the head and posterior wing margins. Significant changes in ventral makings have not been observed in observation periods exceeding 5 years. Scars and missing tissue may be distinctive for an individual, but significant healing may occur in 12 months. The method was designed for identification of chevron colour-type mantas, but is equally useful in identifying black colour-type individuals, and has a wider application for other mantas or marine megafauna taxa including whale sharks and cetaceans.

Key words: *Mantas, scars, ventral markings, visual identification*

Introduction

Until recently, the genus *Manta* had been considered monotypic despite up to 10 generic and 25 specific synonyms being used historically and all research in recent years has been attributed to *Manta birostris* (Walbaum 1792). New research by Marshall et al. (2009) has separated two species based on morphometric measurements and easily identifiable external characteristics including colouration, the presence of a caudal spine in a postdorsal protruberance (*M. birostris* only) and size. The second species is *Manta alfredi* (Krefft 1868), growing to 5 m disc width (DW) compared to 7 m DW in *M. birostris*. Both species occur worldwide in tropical and subtropical regions and *M. birostris* is known to occasionally migrate into temperate waters (Last & Stevens 1994). Despite this worldwide distribution there is very little published information on the distribution or biology of manta (Yano 1998). In order to undertake studies on the migration and behaviour of individual animals it is essential to develop a system for identification which can be used at any site where mantas are regularly observed.

In common with population, migration and ecology studies of other charismatic fauna including whale sharks (*Rhincodon typus* Smith, 1828) and cetaceans, the first step was to develop a reliable method to identify individual animals. Species appear to be identified by different characteristics depending on specific physical characteristics which show variation in the species. Whale sharks are identified by the spot pattern between the gill slits and pectoral fins (Arzoumanian et al. 2005), humpback whales (*Megaptera novaeangliae* (Borowski, 1781)) are identified by the ventral aspect of the tail fluke and left and right aspects of the dorsal fins (Blackmer et al. 2000). In mantas, it appears that each individual has a characteristic pattern of dark markings on the ventral side. This pattern has been used by Mr Takashi Itoh, a professional diver (Ishihara & Homma 1995, Homma et al. 1999) and the late Kazunari Yano (Yano et al. 1998) to identify individual mantas in the Yaeyama

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and Ogasawara Islands, Japan. In addition, Manta Bay Resort in Yap, Micronesia have kept records of the ventral side of the mantas which regularly visit cleaning stations nearby (from Manta Ray Resort website, manta ID programme: www.mantaray.com) as do the Manta Pacific Research Foundation (MPRF), based in Kona, Hawaii (MPRF website: www.mantapacific.org). Although there is a consensus that the markings can be used to aid individual identification, there is no stated method for the process.

The purpose of this paper is to propose a method of visual identification of individuals of manta ray (*M. alfredi*) and storage of these records in a specially designed Access database.

Knowledge on worldwide manta population and migration is important due to the tourism income from snorkelling and diving with mantas. Tourist diving with mantas is established in several places throughout the world where mantas are regularly encountered, notably: Hawaii, Yap, East Africa, Indonesia, and, specifically, the Maldives. Tourism is the largest industry in the Maldives and contributes 28% of the GDP. Approximately 35% of tourists visit the Maldives to dive. Diving with sharks and rays is stated as the primary goal for most diving visitors to the Maldives (Anderson & Waheed 2001), thus diving with mantas is a very important activity as it contributes significantly to the income from tourism. There is no commercial manta fishery in the Maldives, and shark and ray fishing was banned in tourist atolls (Male, Ari, Baa, Lhaviyani) in 1995 to protect the elasmobranchs for the enjoyment of sport divers (Anderson 1998). The fishing ban was extended to all atolls in 2003 when tourist expansion plans were published involving tourist facilities in all atolls. There are currently 88 resorts and 110 registered tourist boats which all provide diving facilities and cater for the growing number of tourists visiting the Maldives. Based on government figures of 600,000 visitors to the Maldives in 2005, if only half of the 35% divers visited a specific manta dive site, this would be 105,000 dives made with mantas at a minimum spend of $35 a head. A spend of $3.675 million to see mantas would be a minimal estimate of the income from mantas, as some resorts charge up to $3000 a head to dive with mantas. This income provides salaries for local divemasters, boat skippers, boat builders, dive centre staff, etc. The diving industry is a major employer of local workers. Despite this great number of casual observations of mantas by divers, prior to this study no systematic population or migration studies had been undertaken in Maldives other than a small-scale manta tagging event (Anderson 1996).

Mantas are known to visit specific sites known as 'Manta Points' or *Madivaru* in the Maldivian Dhivehi language. These sites are cleaning stations close to the outer fringing reefs on the lee side of the atolls to the prevailing monsoon winds. The Maldives has two monsoon seasons: from December to April the wind blows mainly from the north east, so mantas are typically found on the west sides of the atolls; during the May–November period, the winds blow mainly from the south west and mantas are observed on the east of the atolls. The lee side has poorer visibility likely caused by higher concentration of plankton which attract mantas to feed and then visit nearby cleaning stations. This phenomenon is only just being described (Anderson et al. 2008), but the lee side plankton blooms are well known (McClanahan et al. 2000).

Material and methods

Surveys

Between November 2001 and November 2007, 380 surveys were conducted at nine cleaning stations in the central atolls where mantas are regularly observed. These cleaning stations are reef areas where cleaner fish await client species of many varieties (Feder 1966) including mantas. Each site is known as a Manta Point (many are called *Madivaru* in Dhivehi). Based on preliminary surveys, these sites were selected as most likely to produce quality manta sightings. With one exception, surveys were carried out as part of a commercial tourist diving itinerary. The nine primary survey sites are listed in Table I and locations identified in Figure 1. Other sites were visited, but sightings information is not included in this paper.

Each survey consisted of a dive on scuba, for a period of approximately 1 h, to depths ranging between 2.5 and 30 m, with the specific objective of visiting a cleaning station to observe mantas. Data were captured using still cameras (both film and digital), digital video and sketches and notes made on an underwater slate. The latter was necessary due to the difficulty in capturing a photographic or video still of the entire ventral side of the manta being identified, including the genitalia and tail. Size was also recorded. All information was collated after the dive and transferred to record sheets and, latterly, to a specially designed Access database.

Identification

Two distinctive colour variations in *Manta alfredi* species have been identified: chevron and black (Barton 1948). Chevron mantas have a distinctive V-shaped area of pale pigment on the dorsal surface just anterior to the dorsal fin (Figure 2a) and

Table I. Central atoll survey sites, location and results.

Survey site	Locality (atoll)	Depth range (m)	Number of surveys	Number of mantas identified
Lankan	Male	15–20	174	1154
Sunlight	Male	14–18	12	49
Boduhithi	Male	2.5–5	47	556
Fairytale reef	Male	18	4	17
Nelivaru	Baa	12–22	3	3
Table Thila	Ari	16–22	52	575
Kalhahandi Huraa	Ari	15–20	15	30
Madivaru	Ari	12–15	71	291
Maavaru	North Nilandhe	14	3	5
Total			381	2680

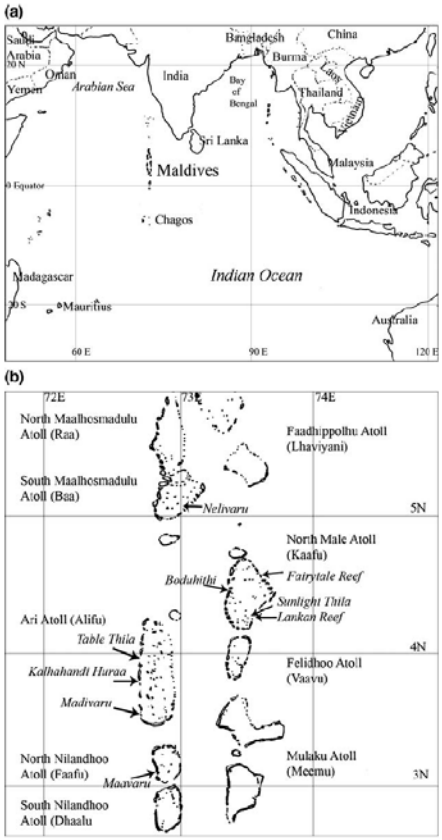

Figure 1. (a). Diagram of Indian Ocean. The Maldives archipelago is located to the south west of India (centre). (b) Diagram of central Maldives atolls to indicate locations of survey sites identified in italics. Atoll outlines indicate fringing reef of depth 1–10 m and not intended to represent land mass. Atolls are named by their geographical name with the administrative name in brackets.

have widely varying dorsal and ventral colour patterns. Black mantas are entirely black on the dorsal side and the majority of the ventral side is darkly pigmented (Figure 2b) except for the areas between the gill slits and the abdominal area posterior to the gill slits, which are white. The vast majority of mantas observed in the Maldives are chevron *Manta alfredi* and the methodology developed here has been primarily developed to identify this species but can be adapted for *Manta birostris* and the black colour morph of both species.

Identification criteria list

In order to achieve a definite identification, the following characters should be recorded:

1. Sex.
2. Disc width.
3. State of tail.
4. Pattern of markings between gill slits.
5. Pattern of distinctive grey mottles and black spots on the ventral surface posterior to the gill slits.
6. Pattern of distinct dark grey patches on distal ends of gill slits.
7. Degree of pigmentation of ventral surface of wings posterior to gill slits.
8. Degree of pigmentation around mouth.
9. Distinctive dorsal pigmentation.
10. Bites (missing tissue) and prominent scars and scratches.
11. Presence of remoras (*Echeneis naucrates* Linnaeus, 1758).

The first three characters can be recorded quickly and should thus be recorded initially. The fourth, fifth and sixth allow progressive narrowing down to a 99% positive identification. The remaining characters provide confirmation. Initially only the first four characters were recorded, but these are found to identify approximately 75% mantas seen. The fifth and sixth characteristics enable all mantas to be identified except those with all-white ventral

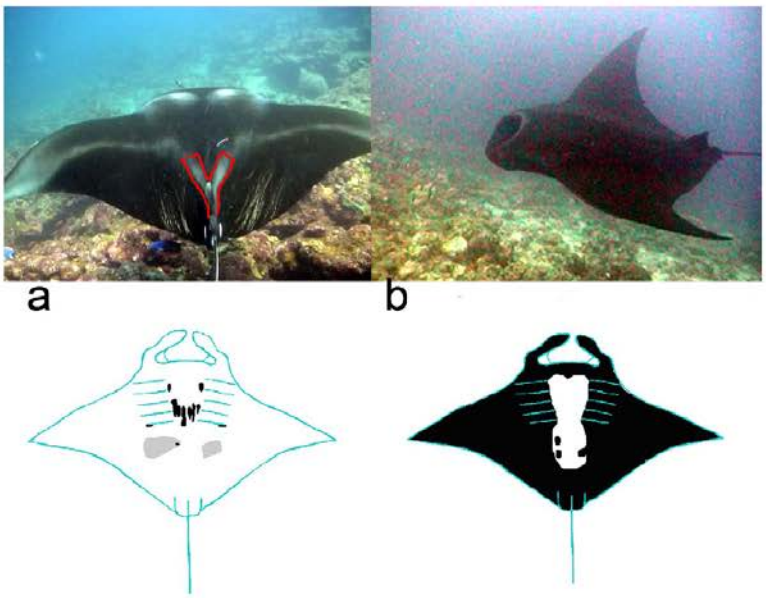

Figure 2. Comparison of dorsal (top) and ventral (bottom) colour patterns of typical chevron (left) and black mantas (right). The photograph (a) shows that the V-shaped chevron (outlined) on the dorsal surface extends laterally to the wing tips as a pale stripe. The diagram (a) (below) shows a mainly white ventral surface. The black manta (b) (right) has an entirely black dorsal surface and the ventral surface is mainly black pigmented. Ventral pigmentation coverage can vary from 50 to 90%.

coloration, which account for less than 1% of the population. This has been confirmed by Guy Stevens (personal communication), another researcher independently identifying mantas from the same population.

1. Sex

Individuals can be sexed visually. The female (Figure 3 left) has a simple cloaca located between the ventral fins; the male (Figure 3, right) has two claspers extending from the pelvic fins. In subadult males these may be relatively undeveloped.

2. Disc width

Disc width (DW) is estimated in proportion to diver size (Figure 4). Mantas were very wary of measuring tapes laid on the reef or in the water column, so calibrating visual estimates was difficult. Size estimation

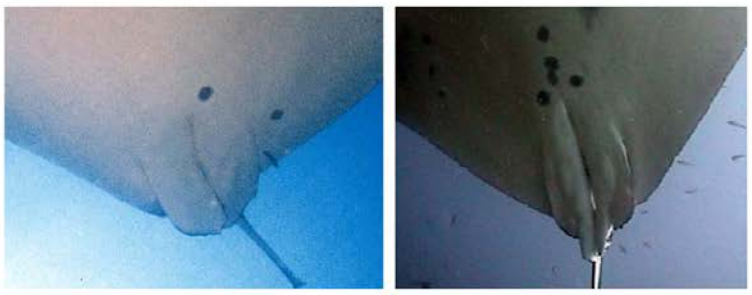

Figure 3. Female genitalia (left), male genitalia (right).

Figure 4. Disc width (DW) may be estimated in proportion to diver size. Diver with fins = 2 m, this manta (*Manta birostris*) is approx. 4m DW. (Photo: Franco Banfi.)

was checked on one survey when a 4 m × 4 m square area was taped off at a cleaning station. An additional five mantas were measured using a tape and compared to the estimated size. It is noted that mantas were resistant to being measured using a tape (it caused significant disruption to their activity and frightened them away) and this is why a visual estimation method was used. For mantas with similar patterns for markings, size can be a useful discriminator.

3. State of tail

Many mantas have damaged tails. Four tail states were recorded.

1. Long intact. No evidence of damage, typically >1 m length.
2. Long cut. The tail end looks damaged – is blunt rather than pointed – but still appears to be at least 75% of the expected intact length (> 0.75 m).
3. Medium. The tail is less than 75% but more than 25% of expected length (approx. 0.5–0.75 m).
4. Short. No tail or less than 25% of expected length (<0.5 m).

Manta tails do not regrow, so a manta first seen with a short tail will not be subsequently seen with a long tail. A long tail can be bitten off, so later sightings could be with a shorter tail.

4. Pattern of markings between gill slits

There is a pattern of dark oval or diamond-shaped (ovoid) marks located in the area between the gill slits. Individuals' patterns changed negligibly over a period of 3–5 years, and so can be used for identification during observation periods within this timescale. This is the most important character for individual identification. Mantas are known to be born with a full markings set (Marshall et al. 2008; Uchida et al. 2008). This pattern will distinguish about 75% of mantas. The more markings present, the more likely that the pattern will uniquely identify an individual. Gill slits are numbered from top (1) to bottom (5) (Figure 8) and described as they are on the animal, not as it appears in the photos i.e. the right gill slits are the manta's right gill slit (left in photos). The most common marking is a single dark oval between the levels of the fourth and fifth gill slits (Figure 5a), but there are so many potential positions for even a single mark that this pattern remains the most useful identifier. When reviewing photographs or stills from video, care must be taken to separate oval markings from cleaner fish. A combination of

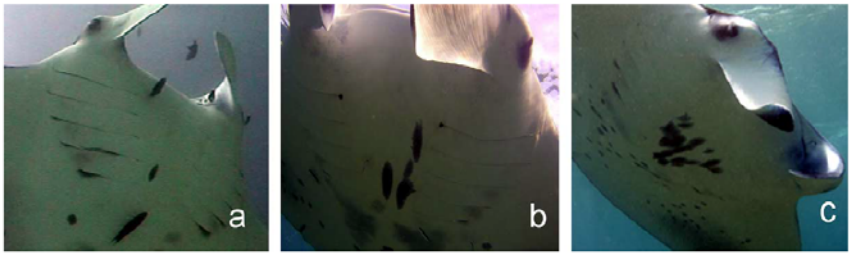

Figure 5. Markings between the gill slits are the most important tool in identifying individual *Manta alfredi*. The marks are typically ovoid and not round in shape. (a) Has one single oval located between the levels of the fourth and fifth gill slits. Two oval cleaner fish are visible, one near the first gill slit and one (*Thalassoma lunare* (Linnaeus, 1758)) posterior to the oval marking. In (b) there is a set of four ovoid marks of varying size, three are in an approximate line adjacent to the left gill slits and there is a single oval centrally in line with the fourth gill slits. In (c), a complex pattern of approximately 19 oval marks create a distinctive 'W' (hence this manta is known as Winifred).

video and sketches on underwater slates is the most useful way of capturing the data. Figure 5a–c shows some examples of progressively more complex patterns between the gill slits. When recording the pattern on a slate, or reviewing photographs or video, it is useful to visualise a notional 20 cell grid using the gill slits as a frame (Figure 6). A very simple pattern may only require an eight-cell grid, whereas a more complicated pattern will require more grid cells to record it effectively. An example of how the grid distinguishes a common pattern is shown in Figure 6. In the first animal (left diagram Figure 6) the mark is in the centre, between gill slits 4 and 5 but extending slightly upwards from gill slit 4 level. For the second animal (right diagram Figure 6) the mark is between gill slits 4 and 5 but is contained within the grid markings and very much to the right of the gill slit area.

Applying a grid to a more complicated pattern to accurately identify where the marks are will normally result in marks being between sections. For the manta in photograph Figure 7, there are five main marks which have been outlined in black to make them easier to identify. The top mark extends the grid above slit 1 and the lowest two extend below slit 5. It is very common to have marks outside the grid. All five marks cross grid sections. The grid helps the eye with accuracy in identifying where the marks are.

Approximately 10% (112 out of 1114 sampled) of mantas observed have no markings between the gill slits so additional marking data is collected and these are the criteria listed 5–11.

5. Pattern of distinctive grey mottles and black spots on the ventral surface posterior to the gill slits

This specifically describes pigmentation in the area immediately posterior to the gill slits on the ventral side on the manta's abdomen. The pattern consists of grey mottle shading covering between 1% and 80% of the lower ventral area and can extend towards the wing tips and anterior into the distal gill slits. Sometimes the mottle shading is absent. Mottle shading varies in colour from light grey to black. In addition, there is often a complicated spots arrangement in this area, but in contrast with the area described in point 4, the black spot marks are always circular rather than ovals or diamonds in a typical chevron manta and typically of diameter <6 cm. Exceptions are discussed at the end of this section. This pattern is a very important identifier, especially when there are no or very few marks between the gill slits. Some examples are shown in Figure 9. The circular spots in the ventral abdominal area can be very distinctive, either in sheer quantity, or in creating specific, eye-catching patterns.

Whilst the vast majority of mantas observed in the Maldives are typical 'chevron' colour type, an occasional 'intermediate' variant is seen with a chevron on the dorsal side but 'black' type ventral markings. These morphs have no markings between the gills slits (this is an identifying factor) and ovals and diamond patterns in the posterior abdominal area. To identify individuals of these types use the criteria 1–5 and record the number and pattern of marks in the area posterior to the gill slits. In common with the marks typically found between the gill slits in chevron mantas, the posterior ventral markings on black and intermediates are almost black in pigmentation, and distinctive patterns are formed from combinations of ovals rather than dots and mottles (see Figure 14 for two examples).

6. Markings on gill slits

This is specific to the dark shading on the distal edge of the actual gill slits. This shading varies significantly between animals and should be recorded especially when the markings between the gill slits are very simple. The variation in makings on the gill slits is very significant and is often proportional to the general level of pigmentation of the animal; thus a pale animal may have no gill slit marks whilst a very dark animal may have every gill slit heavily pigmented. The pattern is recorded as a binary code 0 = no mark, 1 = mark. For each side starting with the gill slit nearest the mouth the marks would be recorded as 1st/2nd/3rd/4th/5th. If no slits were marked the code would be 00000, if all slits 11111. The photograph in Figure 8 has the gill slit marks

Figure 6. Use of a notional grid may assist accuracy in visually identifying location of markings between gill slits. If the grid is applied over the diagrams of similarly marked mantas, the location of the single mark is obviously different in each.

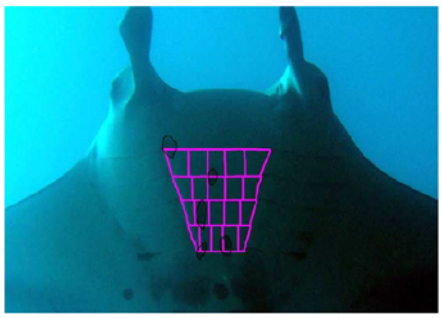

Figure 7. Ovals are outlined and notional grid placed over marks to aid visual location of each mark. There are five ovals in the area identified on this *Manta alfredi*.

outlined and indicates that only the fifth gill slit on both sides have black marks on the outside and would be recorded as 00001 00001.

7. Degree of pigmentation of ventral surface of wings posterior to gill slits

A variation in colouring is looked for on the ventral side of the wings (Figure 10 left vs. right). The area in the posterior half of the wings will be obviously darker than the anterior area and the pigmentation is symmetrical. This is a general indicator of overall pigmentation. Pale mantas will not usually have darkened ventral wings, and black mantas will have the ventral wing pigmentation continue anterior towards and including the head in a single block of pigmentation. Intermediate chevron mantas are very distinctive for this characteristic (see Discussion). The ventral wing area is symmetrical in pattern and highly pigmented and clearly delineated from the non-pigmented area. The pattern and area of pigmentation may vary. At one extreme, the entire posterior ventral area will be pigmented, at the other, only the distal ventral wing margin will be pigmented (Figure 14 shows standardized drawings of the ventral sides of two intermediate mantas).

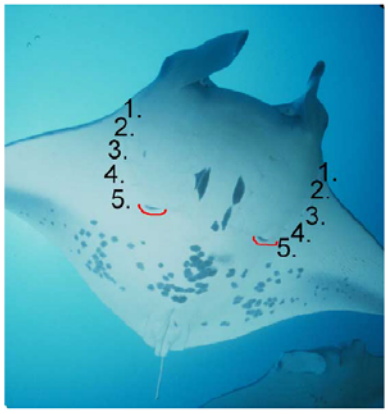

Figure 8. Gill slit numbering etiquette: 1 is top, 5 is bottom. In this example, only the bottom slits on both sides have distal black marks (underlined for ease of identification). This is the most common combination in *Manta alfredi* and is recorded as 00001, 00001 (0 = no mark, 1 = distal mark).

8. Degree of pigmentation around mouth

The degree and shape of this pigmentation can be quite distinctive and is indicative of the overall level of pigmentation in the animal (Figure 11). Cephalic fins are usually white or partially pigmented at the base but some animals the cephalic fins are entirely pigmented in a range of shades from pale grey to black.

9. Distinctive dorsal pigmentation

Dorsal pigmentation varies significantly in chevron mantas with variations creating contrast between dark base colour and pale patterns. Dorsal pigmentation colour varies from pale grey/fawn to black, including shades of brown and purple. The area most likely to be pale (cream/grey to white) in addition to the chevron is the area behind the head, and extending to the eyes, which is referred to as a 'collar' in this document. Predictable dorsal pigmentation may also include white wing tips and white shading extending forward from the chevron to the central dorsal surface and across to the wingtips and the collar which may join up resulting in a pattern with a higher amount of pale area than dark.

The photograph (Figure 12) of the entire dorsal side of a chevron manta clearly shows the chevron, collar and wingtips. There is a faint line connecting the chevron to the wingtips. Black mantas are entirely black on the dorsal surface.

The dorsal surface pattern varies significantly between animals, but is much less distinctive than the ventral pattern. Therefore records of dorsal pigmentation are only made when variation is significant, for example very pale, or entirely black.

10. Bites (missing tissue) and prominent scars and scratches

Many mantas have large areas of lower wings missing from apparent shark bites. These marks are very distinctive. Smaller scars and scratches have been

Figure 9. Spots (black, round marks) and 'mottles' (areas of dark grey shading) in posterior ventral area can be used as secondary characteristics for identification in *Manta alfredi*. Top left: Five spots near genitalia in 'Y' shape; top right: complicated pattern of varying sized spots; bottom left: spots extend all over posterior ventral area; bottom right: distinctive six spot set and otherwise very white with few other marks.

seen to heal over 6–12 month periods, so are less objective for identification.

11. Presence of remoras

Although remoras (*Echeneis naucrates*) can come and go, it appears that once a manta has remoras in association with it, they seem to remain with it. This may be due to the behaviour and migration of the manta.

Field notes

Points 1–4 are essential, and any record with less than three of the essential criteria was immediately excluded. The most important data required is an accurate record of the spots between the gill slits (point 4) and this continues to be the most significant marker used to identify an individual. It was common during an observation period to disregard animals if a full identification set could not be recorded through photos and notes.

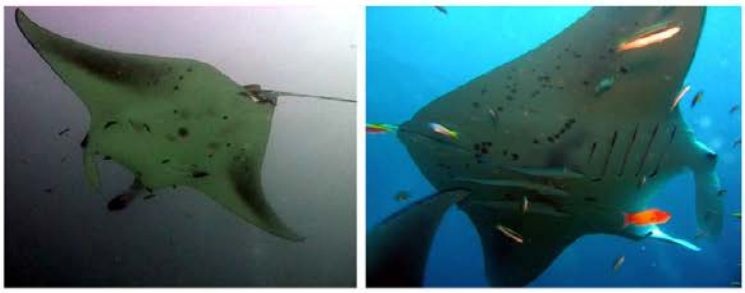

Figure 10. The manta on the left has dark ventral wings, the one on the right: pale ventral wings. Both are chevron *Manta alfredi*.

Figure 11. The manta on the left is completely white around the mouth. This is the most common variety for Maldives mantas. In contrast, the manta on the right has pigmentation extending from the mouth to the eyes creating a 'hood' effect, but the cephalic fin tips remain white this photograph is of an intermediate colour type, likely *Manta birostris*.

The criteria list was developed over a period of less than 3 months and 15 surveys. Observation feedback quickly identified which markings were important and which were secondary. The criteria list was added to and refined as experience in matching increased.

Matching and recording

Matches were made by the author by comparing records. New records would be compared to the standardized drawing of similar existing records using criteria searches in the Access database for sex, number of spots, distal edge of gill slit coloration to create a shortlist of potential matches; a final match would be confirmed by comparison to photographs and digital stills used to create the standardized drawing before a match was confirmed. Certain allowances were made depending on the time between observations. A match of spots and sex was essential. On rare occasions a slight variation in spots would be considered, in particular it was noted that some spots darkened, especially when the mark had originally been recorded as being grey or pale and a period in excess of 12 months had occurred between observations. There was no record of marks disappearing. Size was matched with an allowance for a size increase for observations after six months or more. The maximum size increase was for a male manta observed to increase in disc width by 1 m in 3 years. The majority of animals observed did not increase in disc width or increased by 0.5 m or less. Tail length could only decrease for subsequent observations. If a match was not made, an additional animal record was created.

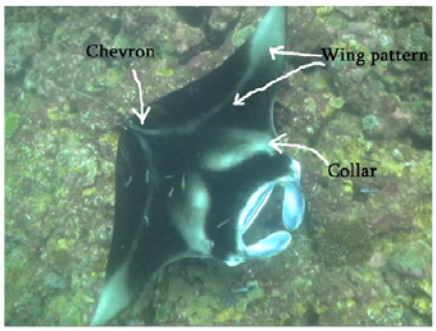

Figure 12. The 'V'-shaped chevron is indicated on the dorsal side of manta. The collar and wing tips on this *Manta alfredi* are pale grey to white. A grey line extends from the chevron to the wing tips. The base colour is very dark grey, almost black.

When animals were matched, a note of the date of the re-sighting was made along with any additional relevant information including behaviours or pregnancy. The drawings on a 500 × 500 pixel GIF format standardized base are the main visual record. Photo and video records are used in support. Each record is allocated an alphanumeric code based on the order of sighting and location first sighted; thus, L23 would correspond to the 23rd manta identified at Lankan survey site and its full database code number of 23 L23 would correspond to it being the 23rd entry in the database. The standardized drawing includes a record of sex and tail length next to a sketch of the markings between the gill slits, distal gill slit pigmentation and identifying marks in the posterior abdominal area, plus distinctive scars or missing tissue. The drawings are intended to help match, not provide a perfect drawing of the ventral markings. The standardized drawings are amended when new information is obtained; for example, new photo or video footage is obtained and a minor detail requires change. In general, the standardized drawings are easy to recognize and other observers of mantas in the Maldives have been able to match the drawings to their own sightings and photo records easily. Figure 13 shows a standardized drawing with its file photograph.

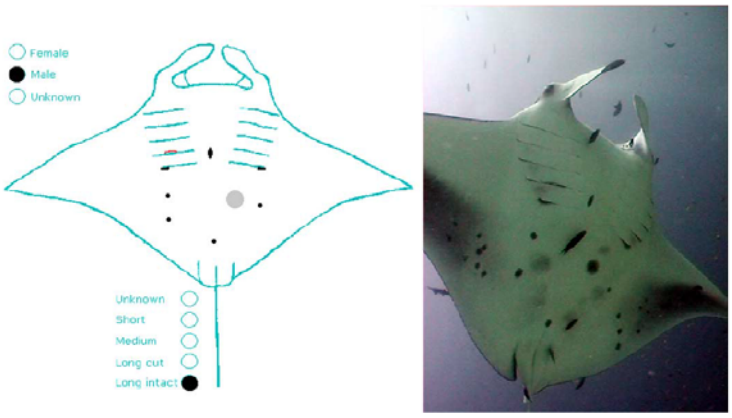

Figure 13. Standardized drawing (left) of *Manta alfredi* 35 S35 from Access database and file copy of good ventral photograph (right). Not all marks are copied from photo to standardized drawing, only characteristic and obvious marks are recorded. The standardized drawing includes the single oval between slightly right of centre between gill slits 4 and 5, four black spots located in the central lower abdominal area plus a grey mottle left of centre on the abdomen. The red line indicates a scar on the fourth right gill slit.

To date, a method for automatically reviewing either the standardised drawings or the photo databases has not been developed, and this is mainly due to the difficulty in establishing markers for an automatic search to start from due to the changing shape of the ventral side as the manta flexes and bends during swimming, plus difficulty in obtaining a ventral photograph with all the physical characteristics required to obtain a match. Based on experience, over 80% of photos would need to be disregarded, which is an unacceptably high loss of data. The described method allows most ventral photographs to be processed.

Results

Table I outlines the survey sites and number of mantas identified at each site. The number of individual mantas positively identified during any survey ranged from 1 to 40 animals from a sample of 1 to over 100 animals observed.

The total number of positive identifications was 2680 from 3013 animals sighted (68.6%).

The total number of individual mantas observed was 1440 (*M. alfredi*: n = 1434; *M. birostris*: n = 6) from 2680 sightings; 987 (68.5%) individuals have only been sighted once.

Of the 302 North Male atoll mantas (mantas seen at sites: Lankan, Sunlight thila, Boduhithi, Fairytale Reef) which have been seen more than once, 164 have been seen at a different site in the atoll to that which they were first recorded at; 146 have been recorded on different sides of the atoll during appropriate monsoons (east or west).

For individual mantas, the reshow varied from zero (seen only once) to 26 re-sightings of manta 243 L218 known as 'Butterfly'. This manta was observed at both Lankan and Boduhithi sites (located on east and west of North Male atoll, respectively) during opposite monsoon periods over a number of years from 2003 to 2007. Observations indicated that she was pregnant during 2005 and 2007 but not in 2006. A full list of sightings and comments for this manta is recorded in Table II.

Only 68 mantas in North Male atoll were seen on five or more occasions (range 5–27). In Ari atoll only 11 mantas (out of 722) were seen on five or more occasions (range 5–8).

This database is the first record of a black manta of either species in the Maldives, reported at Kalhahandi reef in central west Ari atoll on 6.3.2003 (ID number 1001 K12). Along with one other black and four intermediate colour morph individuals these are identified as *M. birostris* from distinctive markings, specifically the lack of marks between the gill slits, ovals in the posterior abdominal area and a clearly delineated margin on the posterior wing margin for the intermediate colour type mantas. Two *M. birostris* standardised drawings are shown in Figure 14.

Size estimates were reviewed. From the calibration exercises, the two animals measured against the tape set in a 4 m × 4 m square were (within ± 30 cm) 4.1 m and 2.5 m DW. The five mantas actually

Table II. Chronological sightings data of manta 245 L218 demonstrating an annual cycle of East to West and back movement across North Male atoll for years 2004–2007 and being reported pregnant in 2005 and 2007.

Lankan (East) sighting of Manta 243 L118	Boduhithi (West) sighting of Manta 243 L118
28.6.2003	
3.7.2003	
22.8.2003	
28.10.2004	
	14.2.2005
4.10.2005 reported pregnant	
6.10.2005 reported pregnant	
15.10.2005 reported pregnant	
2.11.2005 reported pregnant	
5.12.2005 reported not pregnant	
	24.3.2006
	25.3.2006
13.5.2006	
23.5.2006	
27.5.2006	
17.7.2006	
22.7.2006	
26.7.2006	
9.10.2006 reported not pregnant	
	24.2.2007 reported not pregnant
	25.2.2007 obvious mating scar
24.4.2007	
30.4.2007	
28.5.2007	
18.6.2007 reported pregnant	
30.10.2007 reported not pregnant	
4.11.2007	

measured and compared were 0.1–0.5 m greater than visual estimates.

The range of sizes was 1.75–5.0 m ($n=1379$, not all mantas recorded in database had an estimate for size given). Both sexes showed the same range. Overall mean size was 3.052 m (SD 0.556), and median size was 3.000 m.

Mean female size was 3.265 m (SD 0.516) and median female size was 3.250 m ($n=872$), whereas mean male size was 2.692 m, median male size was 2.50 m ($n=507$). A two-sample t-test showed that females were significantly larger than males ($P<0.001$).

Discussion

The identification method has been effective in positively identifying 2680 mantas and creating records of 1440 individuals. Other manta observers (over 70) in the Maldives have been trained to use the method (see Acknowledgements) and contribute to the database. This is the largest database of mantas in the world and continues to grow by approximately 200 new mantas each year. Although the majority of mantas (68.5%) have only been recorded once, new records continue to be added as experience has shown that a manta may be re-identified up to five years after first sighting.

The Maldives population is primarily (>99.5%) comprised of standard chevron *Manta alfredi*. The black and intermediate varieties are very rare in the Maldives (only six individuals recorded). Based on visual evaluation of photographs of mantas from the Eastern Pacific and West Atlantic oceans (Karey Kumli, Robert Rubin, Osmar Luis, personal communications) and research on visual identification of the population in the Revillagigedos Islands (Kumli & Rubin 2008), the black and intermediate mantas

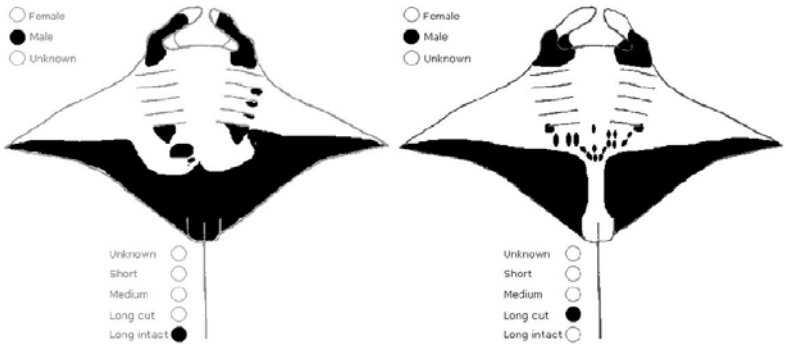

Figure 14. Manta 992 K2 (left) and manta 1211 Q1 (right) are two intermediate mantas in the database. They both have darkly pigmented ventral posterior wing margins and no marks between the gill slits. In the left manta the posterior wing pigmentation includes the genital area; in the right manta the genital area is white. Both have ovoid-shaped marks (not spots) in the abdominal area posterior to the gill slits. These markings are very distinctive and it is likely that these are examples of *Manta birostris*.

observed in the Maldives are likely a different species of *Manta* (cf. Kashiwagi et al. 2008; Marshall et al. 2009), *Manta birostris*. The latter can be distinguished by its larger size (adults grow to >5 m DW) from *M. alfredi* (commonly seen and described from the Maldives, Australia, Yap, Hawaii and East Africa), the presence of a postdorsal protruberance, teeth in both jaws, and distinctive ventral markings as described in this document as 'intermediate' (i.e. no markings in the area between the gill slits but a distinctive pattern of ovals (not spots) in the area posterior to the gill slits and a delineated area of dark pigmentation on the posterior wings margin starting at the wing tips and usually continuing towards (and sometimes including) the genitalia area; see Material and methods). This identification of the two black and four intermediate mantas being likely *Manta birostris* is based on their larger size (range 4.0–5.0 m DW, mean 4.333 m compared to mean Maldives size of 3.052 m DW, SD 0.556) and the distinctive ventral markings. The variations in ventral markings and difference in size means that it is usually possible to identify species from visual evaluations of photographs, video or of animals in the wild.

The records are providing important insights to the ecology of mantas in the Maldives. That 48.3% (146/302) of mantas seen more than once in North Male have been seen on both sides of the atoll in the opposite monsoons provides some evidence of annual migration behaviour, as further demonstrated in the alternate site observations of the frequently observed manta 243 L218 in Table II. Manta 243 L218 is reported only at sites on the lee to the prevailing wind in the corresponding monsoon and is absent from windward sites. As mantas are typically only observed once or twice, further investigation on migration is warranted, ideally using tracking devices which can monitor movements continually over a period of months.

The sightings data of the frequently sighted manta provide evidence of her being pregnant in late summer/autumn 2005 and 2007 and giving birth in late autumn by her being observed not pregnant in December and November, respectively, of those years (from Table II). This provides some evidence of a two-year reproductive cycle for mantas in the wild, despite it now being known that mantas may reproduce immediately after parturition after a 12–13 month gestation (Uchida et al. 2008). These examples indicate that multiple sightings data can contribute considerably to the understanding of an individual animal's behavioural ecology.

The formal methodology proposed here is the first for visual identification of *Manta alfredi*, and whilst specifically designed to identify individuals within the Maldives population, can be used to identify individuals from other populations with similar ventral pigmentation patterns, for example, those in Hawaii, Yap, Bora Bora, Mozambique and Australia. The method can be adapted to include black or intermediate colour type individuals (cf. *M. birostris*) in a local database.

Acknowledgements

Whilst the vast majority of sightings were made by the author with assistance from Matt Kitchen recording the observations on digital video, the significant contributions of data, photographs and video sequences by diving instructors Lisa Allison and Shaff Naeem during 2005–2007 should be recognized. The author would also like to thank all the holidaying diver observers who contributed their time and photographs. The Access database was designed by Dr Alasdair Edwards, Newcastle University.

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EXTENT AND ECONOMIC VALUE OF MANTA RAY WATCHING IN MALDIVES

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Manta rays, *Manta alfredi*, are a major attraction for tourist divers and snorkelers in the Republic of Maldives (central Indian Ocean). The aims of this study were to assess the extent and economic value of manta ray watching in the Maldives, by surveys of tourist numbers at manta diving sites, and from interviews with experienced divers. Ninety-one manta dive sites were identified, where tourists made an estimated 143,000 dives and over 14,000 snorkels annually during 2006–2008. This was estimated to be worth about US$8.1 million per year in direct revenue. The growth of manta ray watching has provided support for both research and conservation in the Maldives. However, there are indications that at the most popular manta dive sites the large numbers of visiting divers and snorkelers may be having a negative impact on manta numbers. There is a need for improved tourist education, and perhaps for regulation of diver numbers at some sites.

Key words: Manta ray; Maldives; Wildlife tourism; Economic value

Introduction

The Republic of Maldives is a small island nation in the tropical Indian Ocean, southwest of India (Fig. 1). The country is composed entirely of coral atolls, of which there are 26, and on which there are some 1,200 small sandy islands. The population numbers about 300,000, roughly one third of whom live on the capital island of Malé, while the remainder are scattered over some 200 inhabited islands. The Maldivian economy is based almost entirely on fisheries and tourism.

Maldivian tourism is, naturally, much influenced by the country's geography. This lends itself to the development of exclusive island resorts, of which there were 94 operating at the end of 2008 (Ministry of Tourism, Arts, and Culture, 2009). In addition, there is a smaller, but thriving "safari boat" sector, with over 140 registered liveaboard vessels currently in operation (Ministry

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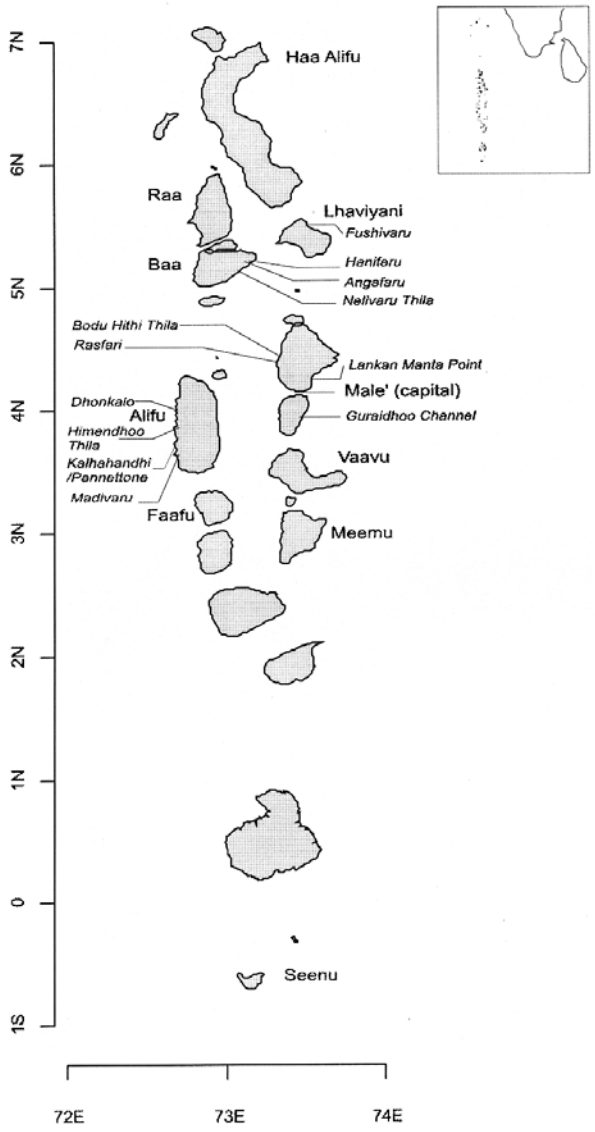

Figure 1. Location map, including positions of major manta diving sites.

of Tourism, Arts, and Culture, 2009). The majority of tourists (75% of arrivals in recent years) come from Europe, with the UK, Italy, and Germany being the largest markets (Ministry of Tourism, Arts, and Culture, 2009). Asia is the next largest regional market, contributing an average of 19% of visitors in recent years (Ministry of Tourism, Arts, and Culture, 2009). Tourism started in 1972 and attracted 683,000 visitors in 2008 (Fig. 2). While the tourist industry grew steadily throughout most of that period, the tsunami of December 26, 2004 had a major impact on tourist arrivals in 2005 and 2006.

The coral reefs of the Maldives are a major attraction for tourists, and diving and snorkeling have always been a key component of Maldivian tourism. While the relative importance of diving has declined in recent years, particularly as more up-market resorts have been developed over the past decade, an estimated 15% of tourists still visit Maldives primarily for diving (Ministry of Tourism and Civil Aviation, 2007). Other tourists also make some dives and many go snorkeling. At the same time the beautiful island and coastal scenery continues to be a major draw. Indeed, there is recognition that the success of Maldivian tourism depends ultimately on the marine environment. As a result, there is awareness of the importance of environmental issues and the need for sustainability within the tourism industry (Ministry of Tourism and Civil Aviation, 2007). More generally, the new *Constitution of the Republic of Maldives*, ratified in August 2008, enshrines the fundamental importance of environmental protection (Ministry of Legal Reform, Information and Arts, 2008, Article 22). The *Maldives' National Biodiversity Strategy and Action Plan* (Ministry of Home Affairs, Housing, and Environment, 2002) emphasizes the importance of economic forces for biodiversity conservation and specifically calls for economic valuation of ecologically and socially important components of biodiversity.

Manta rays, *Manta alfredi* (formerly *Manta birostris*, see Marshall, Compagno, & Bennett, 2009), are a conspicuous and charismatic component of tropical marine biodiversity. In many parts of the world, but not Maldives, manta rays are caught by a variety of fishing gears, but especially gillnets, in which they are easily entangled (Homma, Mauyama, Itoh, Ishihara, & Uchida, 1999; Notobartolo-di-Sciara, 1995; White, Giles, Dharmadi, & Potter, 2006). This and their biological characteristics (including slow growth and small numbers of young) mean that manta ray populations, like those of other chondrichthyan fishes (sharks and rays), can be easily overfished, and once over-

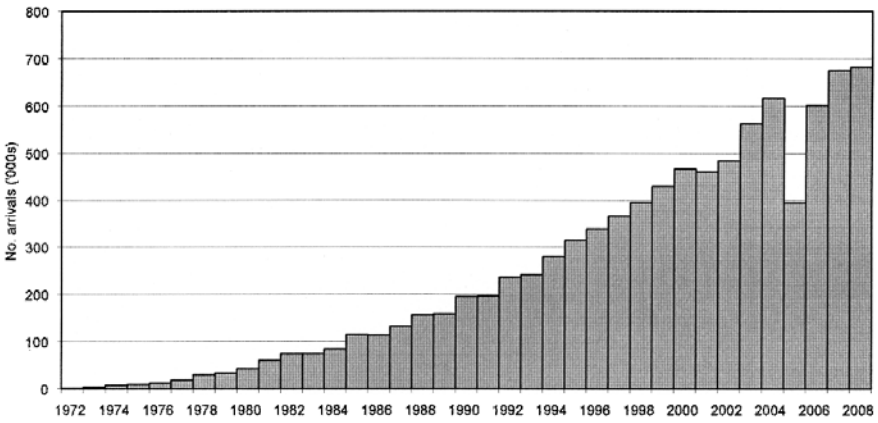

Figure 2. Annual tourist arrivals in the Maldives, 1972–2008 (Ministry of Tourism data, compiled from annual reports).

fished may take decades to recover (Cambi, Fowler, Musick, Brautigam, & Fordham, 1998; Cambi et al., 2009; Dulvy et al., 2008). While manta rays are not thought to be globally endangered (International Union for Conservation of Nature [IUCN] Red List global status Near Threatened), some populations (e.g., around Madagascar, Sri Lanka, India, Indonesia, Philippines, and parts of Mexico) have been heavily fished and may be vulnerable to local extinction (Cambi et al., 2009; Dulvy et al., 2008; IUCN, 2007; Ishihara, 2005; Marshall et al., 2006). Ironically, manta rays are often not even highly prized as food fish (although their gill rakers have recently started to attract high prices for use in Chinese medicine and cuisine). Their meat is often sold at low prices, used as shark bait, or discarded.

In contrast to many fishermen, divers prize manta rays. Divers delight in seeing mantas in their natural environment, and will pay good money to do so. In some areas manta rays occur in regular concentrations, and have become tourist attractions (e.g., off Mozambique, parts of Indonesia, Australia, Philippines, Yap in Micronesia, southern Japan, Hawaii, and Mexico). Because some of these concentrations occur in seas adjacent to relatively poor tropical countries, manta ray tourism may potentially make a major contribution to local economies (Homma et al., 1999), as does shark diving tourism (Anderson & Ahmed, 1993; Norman & Catlin, 2007; Topelko & Dearden, 2005). Furthermore, the economic value of ray and shark tourism may in some cases contribute towards marine research and conservation (Anderson, 2002; Anderson & Waheed, 2001; Burgess, 2005; Newman, Medcraft, & Colman, 2002; Topelko & Dearden, 2005).

In the Maldives, manta rays are relatively common and are a major attraction for tourist divers. Manta ray dive sites feature prominently in Maldivian diving guidebooks (Amsler, 1994; Godfrey, 1996; Harwood & Bryning, 1998), and the Maldives are increasingly described as the "islands of the mantas" (or similar) in advertisements aimed at tourist divers. Waheed (1998) estimated (from a survey of tourists' willingness to pay) that the *potential* value of manta ray watching in the Maldives was about US$7.8 million in 1997. That contingent valuation undoubtedly overestimated the actual value of manta ray diving at that time because the estimate of the extra amount that tourists were willing to pay to take part in one manta dive was applied to all dives. Nevertheless, it is clear that manta ray watching was worth a substantial amount at that time. Waheed (1998) also noted that tourists reported being willing to pay a higher surcharge to see manta rays than to see either sharks or turtles (US$12.80 per dive, vs. US$11.80 and US$10.50, respectively).

The aim of this study is to document for the first time the actual extent and value of manta ray watching by tourist divers and snorkelers in the Maldives.

Method

Study Area

The Maldives archipelago runs north–south from about 7°N to 0.5°S, a total distance of over 800 km (Fig. 1). Until very recently, tourism was confined to the central tourism zone (southern Raa and Lhaviyani Atolls in the north to Dhaalu and Meemu Atolls in the south) plus Haa Alifu Atoll in the far north and Seenu Atoll in the far south. This constitutes approximately 55% of the total atoll area of the Maldives. New resorts opened in all the remaining atolls during 2009–2010, but this study was confined to the more restricted area open to tourism up to 2008.

The oceanography of the Maldives is strongly influenced by the seasonal monsoons. The northeast (NE or boreal winter) monsoon lasts from about December to March, during which time ocean currents are predominantly to the west. The southwest (SW or boreal summer) monsoon lasts from about May to October, during which time ocean currents are predominantly to the east. Within the Maldives, the distribution of mantas is known to be highly seasonal, with mantas typically being present at sites on the downstream sides of the atolls; that is, on the western sides of the atolls during the NE monsoon and on the eastern sides of the atolls during the SW monsoon (Anderson, Adam, & Goes, in press).

Data Sources

Information on the extent and economic value of manta ray watching was obtained from two

sources: (1) from interviews with experienced divers, and (2) from personal observations. Some information was obtained during 2003–2004, but the tsunami of December 2004 disrupted tourism in the Maldives, and all economic activities were impacted. As a result this study was interrupted; it was restarted in early 2006 and completed in June 2008. Information on manta seasonality collected prior to 2006 was retained, but all economic data were recollected.

To obtain information from divers, interviews were conducted with experienced resort dive center staff and safari boat dive guides ($n = 52$), who between them had knowledge of every atoll then open to tourism. Interviews were conducted with base leaders, or the next most experienced person available; interviewees had between 4 and 30 years of full-time diving experience in the Maldives, with most having between 8 and 15 years. Most interviews were conducted in person ($n = 38$), but some were conducted by phone ($n = 14$). Divers were asked to identify all manta dive sites that they used and the months during which mantas were present at those sites. Divers were also asked to give additional information for the one or two manta dive sites that they visited most frequently: cost per dive, frequency of visits (i.e., number of boats per week or month), average numbers of divers per boat, numbers of other diving and snorkeling boats seen at the site(s), and seasonal variations.

Our personal observations between January 2006 and June 2008 included 404 manta dives and 180 manta snorkels at 23 different sites. Numbers of boats were recorded and numbers of divers estimated during each visit; on most occasions visits were of 2–3 hours' duration, but 25 full day counts were made. At the time this article was drafted, the authors had a combined 39 years diving experience in the Maldives, during 27 of which mantas were a particular research interest.

Analysis

For this study we consider only specific dive sites where mantas are a particular attraction. For all such sites, total numbers of dive boats and divers visiting were estimated for the season during which mantas were present (usually 4–6 months; periods at the beginning and end of the season when mantas are sometimes but not always present were not included). Estimated numbers of dives per season were multiplied by the estimated average cost per dive for that site to give seasonal diving revenue for each site. Dive prices mostly varied between US$40 and US$85, depending on dive package (a single dive costs more than one of a multidive package) and operator (dives on liveaboard safari boats are usually included within the holiday price and so are difficult to cost individually, but were estimated conservatively at US$40; at the other extreme, one manta dive from a top resort as part of an exclusive day excursion could cost US$750). Sometimes surcharges are applied if special full day excursions were required to visit distant manta sites. Prices used here include boat costs (because everyone must use a boat) but exclude gear hire (because many divers have their own). Average dive prices of US$45 to US$70 were applied to different sites, the lower values being for sites most frequented by liveaboard safari boats, while the higher values applied to sites used mainly by top-end resorts.

Snorkeler numbers and revenue were estimated only for those few sites where snorkeling with mantas is a major activity; at many other manta sites some snorkeling does take place, but it has not been quantified. Snorkeler numbers were estimated in a similar way to diver numbers. An average snorkel excursion cost of US$20 was roughly estimated from information received from interviews and additional ($n = 9$) inquiries at resorts (range from free of charge to US$50, mostly US$15–25).

Results

Extent and Value of Manta Watching

A total of 91 specific manta ray dive sites were identified; these were distributed throughout the entire area where tourism was allowed (Fig.3). Snorkeling is a significant activity at 10 of these sites (where the mantas occur on reefs shallow enough to be clearly visible from the surface).

The extent and value of manta ray watching by divers is summarized in Table 1 and by snorkelers in Table 2. It is estimated that on average some 143,000 manta ray dives and at least 14,000 manta

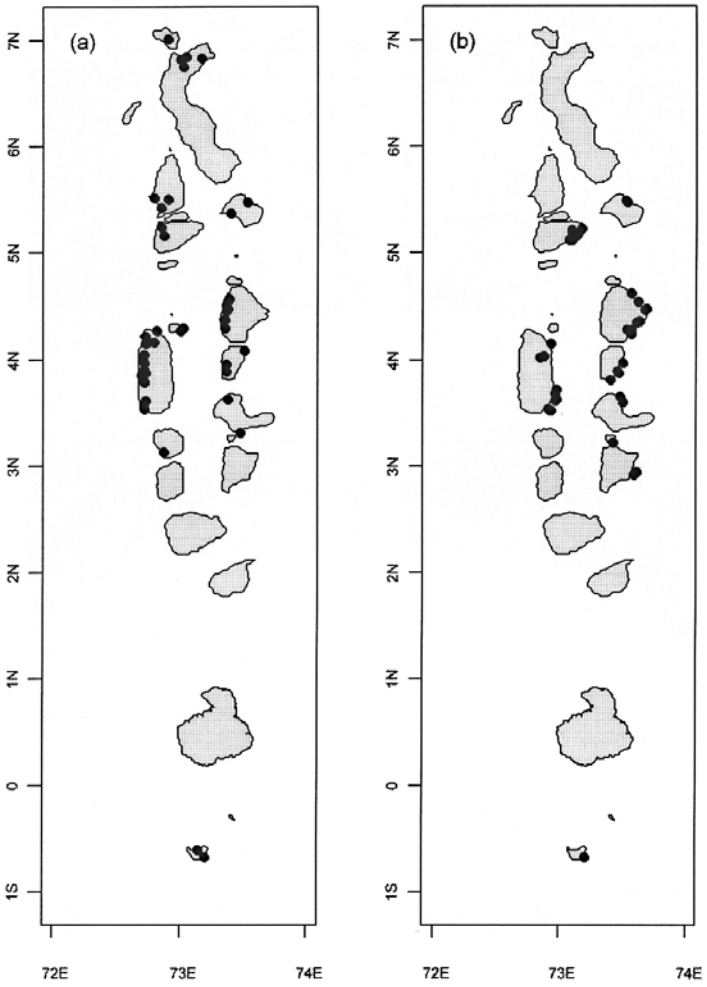

Figure 3. Seasonal distribution of manta rays as reported by divers (each dot represents one dive site, at which mantas are reported to be present in that season). (a) NE monsoon season. (b) SW monsoon season.

Table 1
Estimated Annual Extent and Value (US$) of Manta Ray Diving by Atoll

Atoll	No. Sites	No. Boats	No. Divers	Value
Haa Alifu	5	210	1,700	$119,000
Raa	5	132	1,188	$58,320
Baa	12	2,411	19,230	$1,087,550
Lhaviyani	3	442	5,310	$269,550
North Malé	18	3,423	36,231	$1,798,700
South Malé	9	998	11,068	$602,900
North Alifu	13	1,183	14,796	$880,560
South Alifu	15	3,770	44,840	$2,494,200
Vaavu	6	685	7,045	$409,275
Meemu	2	90	810	$40,500
Faafu	1	32	256	$14,080
Seenu	2	60	480	$26,400
Total	91	13,436	142,954	$7,801,035

ray snorkels were made per year during 2006–2008. This was worth an estimated US$8.1 million per year in direct diving and snorkeling revenue. Total revenue, including accommodation and food, has not been estimated, but must be much more. Major manta ray sites (those generating an estimated annual revenue in excess of US$200,000) are listed in Table 3, and can be located in Figure 1.

Accuracy of Results

The estimates of the extent and value of manta watching presented here do demonstrate the scale of economic interest in this tourist activity. However, we recognize that these estimates may not be especially accurate. In particular, estimating diver and snorkeler numbers by multiplying up number of boats per month by average number of occupants per boat and then again by average cost per participant may tend to produce overestimates. With this in mind, we have been conservative in our approach. In estimating site averages we have ignored the highest reports of boat numbers, divers (and snorkelers) per boat, and cost per dive. And we have not included: diver numbers at the beginning and end of each season, when mantas may not always be present; all dive data from four recognized manta sites where mantas are present for only part of the season; and snorkel data from any but the most popular manta snorkeling sites.

Table 2
Estimated Annual Extent and Value (US$) of Manta Ray Snorkeling by Atoll

Atoll	No. Sites	No. Boats	No. Snorkelers	Value
Baa	2	200	2,000	$40,000
North Malé	3	335	4,020	$80,400
South Malé	2	310	3,560	$71,200
South Alifu	3	420	4,440	$88,800
Total	10	1,265	14,020	$280,400

As a rough check on the accuracy of our results, we note that 683,000 tourists visited the Maldives in 2008 (Ministry of Tourism, Arts, and Culture, 2009), that the average duration of stay was 8.8 days (Ministry of Tourism, Arts, and Culture, 2009), and that 15% of arrivals come primarily for diving (Ministry of Tourism and Civil Aviation, 2007). Assuming that these dedicated divers make an average of 15 dives each, and that other visitors make an average of 0.25 dives each, then a total of about 1.7 million dives were made in Maldives in 2008. With our estimated annual average of 143,000 manta dives, this implies that something of the order of 1 dive in 12 was a manta dive. Resort-based divers typically make about 12 dives per week, and about one manta dive per week during the season when mantas are present in the vicinity of their resort, plus occasion excursions to more distant manta sites during the other season, so roughly 1 dive in 20 might be a manta dive. Liveaboard divers typically make 18 dives per week, and about 2 manta dives per week, so roughly 1 dive in 9 would be a manta dive. Our average estimate of 1 manta dive in 12 is therefore within reasonable bounds.

As a second check on our results, we note that a more detailed study of one manta dive site (Hanifaru in Baa Atoll) in 2009 estimated total seasonal revenue of about US$330,000 (Stevens, unpublished data). Because diver and snorkeler visits to this particular site are known to be increasing, our estimate of US$241,000 per year for the period 2006–2008 appears reasonable.

Discussion

Extent of Manta Watching

A total of 91 dive sites were identified where manta ray watching is a significant attraction for divers and snorkelers. Manta diving sites occur

Table 3
Major Manta Ray Diving and Snorkeling Sites (Value in US$)

	Atoll	Site	Main Season	Annual Value
1	South Alifu	Madivaru	NE	$1,164,800
2	North Malé	Lankan Manta Point	SW	$766,500
3	South Malé	Guraidhoo Channel	SW	$609,300
4	North Malé	Bodu Hithi Thila	NE	$588,600
5	South Alifu	Kalhahandi/Panettone	NE	$547,200
6	North Alifu	Dhonkalo	NE	$468,000
7	South Alifu	Himendhoo Thila	NE	$432,000
8	Baa	Hanifaru	SW	$241,000
9	Baa	Angafaru	SW	$240,000
10	Lhaviyani	Fushifaru	SW (&NE)	$229,950
11	Baa	Nelivaru Thila	SW	$227,500

throughout the area open to tourism up to 2008. That comprised some 55% of the total atoll area, so it is to be expected that many more manta diving sites will be developed in the coming years as tourism spreads to every atoll in the country. It was estimated that during 2006–2008 an average of some 143,000 manta ray dives and at least 14,000 manta ray snorkels were made annually. We are not aware of any other published estimates of numbers of manta ray dives for any other country that would allow comparison. But clearly these are not insignificant figures.

In other areas where manta ray diving is carried out, the number of separate manta dive sites is relatively small. In addition, in other areas where mantas have been studied, the local populations have generally been estimated to be of the order of 50–350 individuals (Marshall et al., 2006). In contrast, the Maldives is home to thousands of manta rays (authors' personal observations and unpublished data). It seems clear that Maldives supports a particularly large population of manta rays, and this in turn supports a particularly large manta watching industry.

Value of Manta Watching

We estimate that diving and snorkeling with manta rays was worth about US$8.1 million per year in direct revenue during 2006–2008.

Because there is little or no market for manta rays in the Maldives, it is not possible to estimate their local fisheries value. However, manta rays are caught and sold elsewhere in south Asia, normally at low prices. In western Pakistan in 2002, manta rays were sold for up to Pakistani Rupees 1500 (about US$22) depending on size (Ali, Arshad, & Akhtar, 2002). In Sri Lanka, mantas are taken as by-catch in gillnets, and occasionally harpooned; catches are believed to have declined over the past decade (Leslie Joseph, personal comments, July 2008). In Sri Lanka in July 2008, manta meat was selling for about Rs 135 per kg (about US$1.25) wholesale, and Rs 240–320 per kg (US$2.25–3.00) retail (Leslie Joseph and Asha de Vos, personal comments, July 2008). Even at the highest Sri Lankan retail prices, a 100-kg manta would be worth just US$300; fishermen would likely receive less than half this amount. A rapidly developing market for manta gill rakers for use in Chinese medicine and cuisine may inflate market prices, but it is still the case that prices paid to fishermen are relatively low.

From photo-identification analysis it is known that several hundred manta rays regularly frequent Lankan Manta Point in North Malé Atoll, but that roughly 75% of sightings are from a core group of about 120 individuals (A.-M. Kitchen-Wheeler and G. Stevens, unpublished data). Because it is the regular occurrence of mantas that makes this dive site so attractive, it could be argued that these 120 individuals are responsible for 75% of revenue. Because this site generates over US$760,000 per year (Table 3), this suggests that each one of these mantas is worth something over US$4,700 per year in terms of diving revenue generated. But

manta rays live for many years (Homma et al., 1999). One individual female has been individually identified as part of a photo-identification project many times at Lankan Manta Point over 20 years, between 1989 and 2009 (G. Stevens, unpublished data). Thus, the life time value of individual mantas in Maldives may be 20 or more times their annual worth, perhaps something of the order of US$100,000 at current prices. In contrast, a dead manta is likely to be worth much less than US$500 elsewhere in south Asia.

Such comparisons make the point that mantas can be worth very much more alive than dead, but they are simplistic. For example, they take no account of displacement effects: if tourists spend their money on diving with mantas, they may have less money to spend on other activities or purchases. They also fail to consider the distribution of revenue. Thus, revenues from fish catches accrue not only to fishermen but also to many others involved in the fishing industry (Chen & Phipps, 2002). Furthermore, diving revenue does not multiply in the way that fish sales do (from fisherman to wholesaler to retailer), and much of it leaks away from local communities (although the diving industry does employ many Maldivians).

With this type of situation in mind, Rodriguez-Dowdell, Enriquez-Andrade, and Cardenas-Torres (2007) have argued, in the case of whale shark watching in Mexico, for the concession of property rights to local operators in order to maximize benefits to local communities. Such an approach is unlikely to find much support in the Maldives, where marine resources are traditionally seen as open access. Nevertheless, the equable distribution of benefits from diving, and indeed tourism in general, remains an issue of concern in the Maldives (Ministry of Tourism and Civil Aviation, 2007).

Manta Ray Protection

Despite these concerns, the wide (but previously unquantified) recognition of the value of manta rays to Maldivian diving tourism has contributed to their protection.

Even without any formal economic investigations, it was recognized in the mid-1990s that manta rays were a valuable resource for tourism (R. C. Anderson, personal observations). As a direct result, the export of all rays was banned from June 24, 1995. Subsequently, the export of ray skins was specifically banned from January 1, 1996 (in response to an attempt by a local company to start ray skin exports). Other protection has come in the form of, mostly small, marine protected areas, of which there are currently 32 in the Maldives. Five of these were designated specifically because of the seasonal presence of mantas (Table 4). However, a more effective form of protection for manta rays in the Maldives is indirect: most types of net fishing (including pelagic gillnetting, trawling, and purse seining) have long been banned, to protect the interests of the traditional pole and line tuna fishermen.

At the time when the export bans were introduced, in 1995–1996, it would have been possible to introduce a total ban on ray catching. That was not done in recognition of the traditional rights of fishermen (R. C. Anderson, personal observations). Manta rays were traditionally caught in small numbers in the Maldives, mainly by harpoon. The largest single cause of fishing mortality was probably the small but regular catch of manta rays by harpoon for use as bait in a specialized fishery for tiger sharks, *Galeocerdo cuvier* (Anderson & Ahmed, 1993). Large tiger sharks were targeted for their enormous livers, which yielded large quantities of oil needed for treating wooden fishing boats. That fishery died out in the 1960s, when more efficient long-lining was introduced. Small numbers of manta rays (and stingrays) have continued to been taken for oil, bait, and skins.

Although local fishermen may still take some manta rays, the ban on exports has had the desired effect of forestalling the development of a major fishery. This provides an instructive comparison with reef sharks, which have also been a major attraction for tourist divers in the Maldives (Anderson, 2002; Anderson & Ahmed, 1993; Anderson & Waheed, 2001; Godfrey, 1996; Harwood & Bryning, 1998).

As with mantas, there is little demand for shark products within the Maldives. But there is a fishery for reef sharks, with the high international price of shark fins driving demand, and most shark catches being exported. In the early 1990s, valuation of shark watching by tourist divers in the

Table 4
Protected Dive Sites at Which Manta Rays Are a Significant Attraction

Atoll	Site	Area	Date Established
Baa	Hanifaru	303 Ha	June 6, 2009
Lhaviyani	Fushifaru Thila	4 Ha	October 1, 1995
North Malé	Rasfari	835 Ha	October 1, 1995
South Malé	Guraidhoo Channel	88 Ha	October 1, 1995
South Alifu	Madivaru (=Faruhuruvalhi)	60 Ha	October 21, 1999

Maldives (Anderson & Ahmed, 1993) demonstrated that reef sharks were worth very much more alive as tourist attractions than dead on a fishing boat. That finding directly influenced government decisions to initiate marine protected areas in 1995 and subsequently to ban all shark fishing in the central tourism zone in 1998. These management initiatives have not been particularly successful in conserving reef shark stocks (Anderson, 1998; Anderson & Waheed, 1999, 2001). However, they did probably slow the decline of reef shark numbers and provided the foundation for a complete ban on reef shark fishing from March 1, 2009, to be followed in 2010 by a complete ban on all shark fishing and shark product exports.

Despite an awareness of the value of shark diving to the national economy, and the introduction of conservation measures (including the declaration of marine protected areas and a ban of shark fishing in some atolls), reef shark numbers continued to decline and reef shark numbers are very much less now than they were in the 1980s and early 1990s (Anderson, 1998; Anderson & Waheed, 1999; personal observations). Without the precautionary ban on ray exports, which was introduced in direct recognition of the value of manta rays to tourism, it is likely that Maldivian manta rays would have suffered the same fate as the reef sharks.

In addition to contributing to their protection, the wide recognition of the value of manta rays to diving tourism also contributes to research on their biology. Many tourist divers visit Maldives in the specific expectation of seeing mantas. Two of this article's authors (A.-M. Kitchen-Wheeler & G. Stevens) are employed by tourism companies. Their employers recognize the attraction of mantas for divers and the value of offering well-informed manta diving and snorkeling excursions. These companies (and others) use mantas in their marketing, and they have facilitated these authors' manta research. This research is improving understanding of manta ray biology, with consequently improved opportunities for divers and snorkelers to have successful encounters with mantas.

Negative Impacts

Wildlife tourism, both marine and terrestrial, can potentially bring benefits for both animals (in terms of better conservation measures) and local communities (e.g., in terms of improved employment opportunities). However, it is clear not only that these potential benefits are often not fully realized but also that wildlife tourism can itself have negative impacts (Cater & Cater, 2007; Garrod & Wilson, 2003; Higham & Lück, 2007; Newsome, Dowling, & Moore, 2005). Despite this, research on tourism impacts is often lacking or insufficient to inform policy decisions and management actions (Lück & Higham, 2007; Rodger & Calver, 2005). This is particularly the case for marine wildlife tourism. But where such detailed information is available, and demonstrates unsustainable practices, it can influence political decisions to manage tourist activities (Higham & Bejder, 2008).

In the case of manta rays in the Maldives, this study was not designed to address issues of tourism impacts. However, it is clear from our own observations, and from interviews with some experienced dive operators, that there are issues of concern. In contrast, several other dive operators appeared unaware or unconcerned about potential impacts of their activities on the manta rays. During interviews some divers pointedly referred to manta watching as a form of ecotourism, which was implied to be a "good thing." To the extent

that manta watching involves observing wild animals in their natural habitat, and has contributed to manta conservation, this may be true. However, most definitions of ecotourism also note that it should have no negative impact on the environment or the animals being observed, and that it should include some element of education (e.g., Cater & Cater, 2007; Garrod & Wilson, 2003; Higham & Lück, 2007; Newsome et al., 2005). In our experience, these last two requirements are often not met in the Maldives.

At the most popular manta dive sites it is quite common to have several dive boats visiting at the same time. On occasion there can be 10 or more boats present and over 100 divers and snorkelers in the water at once. This raises questions about diver and snorkeler safety, and about impacts on the mantas. During the NE monsoon season in 2007–2008, remarkably small numbers of manta rays were seen at many normally productive manta dive sites. The reasons for this are unknown. Nevertheless, we feel, and some experienced divers also suggested, that the large numbers of tourist divers and snorkelers visiting these sites could have caused the mantas to move elsewhere. An alternative hypothesis proposed by other divers was that an unknown change in oceanographic conditions caused the mantas to desert these sites. Because good numbers of mantas were seen at several little-visited sites, both within and without the main tourism area (personal observations), the former explanation seems more likely. (As a counterexample, some individual mantas seem to actively search out interaction with divers—for example, repeatedly "playing" in divers' exhaust bubbles.) While anecdotal accounts such as this are intriguing, the lack of research on diver impacts on mantas does need to be rectified. If, as seems likely, excessive numbers of divers and snorkelers at some sites are affecting manta ray behavior and numbers, then the long-term impact of this disturbance needs to be assessed, and appropriate management strategies introduced.

One factor contributing to the negative impacts of divers on manta rays at some sites may be a lack of tourist education. While all dive operators do brief clients before dives, these briefings are often inadequate. For example, even though most divers are told not to touch mantas, some still do so. More generally, dive operators themselves do not always appear fully aware of the need to behave sensitively towards the mantas. Some dive operators do provide very well-informed briefings before manta dives (and in a few cases even offer specialty manta diving courses). However, in our experience the majority of dive operators in Maldives provide little information about mantas before their dives. Most divers and snorkelers do come away from their encounters with manta rays with feelings of contentment, exhilaration, and even awe, but with little or no additional knowledge to inform their experience. In other areas of marine wildlife watching, notably cetacean watching, onboard education and interpretation are seen as powerful tools to improve tourist understanding and satisfaction, to minimize disappointment if expectations are not met, and to promote conservation (e.g., Andersen & Miller, 2006; Zeppel & Mulion, 2008). This too is an area where manta watching in the Maldives could be improved.

Conclusions

In summary, an estimated US$8.1 million was spent annually on diving and snorkeling excursions to see manta rays in the Maldives during 2006–2008. The high value of manta rays, and of other large marine animals, contributes not only to the development of marine tourism within the Maldives, but also to both research and conservation. Nevertheless, there are indications that at the most popular manta dive sites, the large numbers of visiting divers and snorkelers are having a negative impact on manta numbers. Tourist education needs to be improved, and regulation of diver numbers at some sites may be necessary.

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Biographical Notes

R. Charles Anderson, Ph.D., is a marine biologist and has been resident in the Maldives since 1983. With Maldivian colleague Hudha Ahmed he conducted the first survey of the value of shark watching by tourist divers in 1992. He has been a member of the IUCN Shark Specialist Group since 1993, and started research on Maldivian mantas in 1996.

M. Shiham Adam, Ph.D., studied in the UK and was post-doctoral fellow at the University of Hawaii at Manoa from 2000 to 2003, modeling ocean-scale movements of pelagic fishes. Since returning to Maldives in 2003, he has worked at the national Marine Research Centre. He is now Director General, managing both fisheries and coral reef research.

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Guy Stevens is Senior Marine Biologist for Four Seasons Resorts in the Maldives and the founder of the Maldivian Manta Ray Project. This nonprofit organization aims to further the research on, conservation of, and education about manta rays. Guy is planning to start work on a Ph.D. shortly, using his Maldivian manta data.

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Population estimates of Alfred mantas (*Manta alfredi*) in central Maldives atolls: North Male, Ari and Baa

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Abstract The Alfred manta, *Manta alfredi*, is economically important in the Maldives due to the large number of tourists attracted to SCUBA dive and snorkel with them. It is important to understand the size of populations around main diving points and throughout the Maldives so that manta based tourism activities can be developed more successfully and the impact of tourism can be monitored. Using natural markings to identify individual manta rays, Petersen and Jolly-Seber probabilistic estimators were used to assess population size for main dive sites and the central atolls of North Male, Ari and Baa. Between 2000 and 2009, 1835 individual mantas were identified from 3373 sightings. There was a significant gender bias with a male: female ratio of 1:1.8. Only 24.7% of manta rays were re-sighted between 1 and 34 times with a maximum gap of 7 years and 11 months between sightings. Estimates for populations around main dive sites were 181–562. The estimated population of *M. alfredi* for North Male atoll is 537 (SE±53.2). Estimated atoll populations were extrapolated to obtain an estimate of *Manta alfredi* population for the entire Maldives of 9,677 individuals.

Keywords Mobulinae · Sight-resight · Visual identification · Photo ID · Eco-tourism · Dive industry

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Introduction

Estimates of fish abundance are essential for the assessment and successful management of fish populations (Zeller and Russ 2000). Manta rays are not targeted by fisheries in the Maldives, but they are commercially fished in many parts of the world, such as in Indonesia, India and in the Philippines (Compagno and Last 1999; Homma et al. 1999; Alava et al. 2002; Marshall et al. 2006). The genus *Manta* has only recently been separated into two (or more) species: *Manta alfredi* (Krefft 1868) and *M. birostris* (Walbaum 1792) (Marshall et al. 2009). Two recent studies have estimated local populations of *M. alfredi*. Marshall et al. (2011) obtained annual populations of 149–454 individuals during a four-year study at a site in southern Mozambique and Deakos et al. (2011) estimated that up to 230 animals (from 290 individuals identified over 5 years) were using their study aggregation site in Maui, Hawaii, during 3–4 month sampling periods. There are

currently no estimates of populations of *M. birostris* at any site.

Manta rays (genus *Manta*) are pelagic planktivores and are distributed circumglobally in tropical and warm temperate waters, occurring from 36°S to 40°N (Bigelow and Schroeder 1953; Smith and Smith 1966; Compagno et al. 1989; Michael 1993; Last and Stevens 1994; Compagno 1999; Homma et al. 1999). Manta rays are reef-associated, oceanodromous and pelagic (Riede 2004; Marshall et al. 2009), spending little time near the bottom. *Manta alfredi* is reliably reported from Bora Bora, French Polynesia (Moeava de Rosemont, pers. comm.), Australia, Hawaii, Yap, Maldives, Indonesia, Japan, South Africa and Mozambique (Whitley 1936; Homma et al. 1999; Anderson 2005; Dewar et al. 2008; Marshall et al. 2008; Marshall et al. 2009). Sightings from the Atlantic are very rare.

Manta alfredi is the most commonly seen mobulid species in the Maldives and their high number attract many tourists from around the world (Anderson et al. 2010; Kitchen-Wheeler 2010). It has been shown at several locations that large-bodied, charismatic marine animals generate more money through dive tourism, than having their terminal value from fishing (Anderson 1998; Topelko and Dearden 2005). Ecotourism focusing on whale sharks generate Aus$4.7 million in Ningaloo Marine Park, Australia annually and US$4.99 million in the Seychelles (Davis et al. 1997; Rowat and Engelhardt 2007). Diving with manta rays is a very popular tourist activity at locations where they occur reliably, e.g. Hawaii, Yap, East Africa, Eastern and Western Australia and specifically, the Maldives. In 2006–2008, approximately 143 000 scuba dives and 14 000 snorkel dives were made with manta rays in the Maldives each year generating an estimated US$8.1 million annual income which delivers substantial benefits to the local people of the islands who work as dive guides and boat crews (Anderson et al. 2010). The three atolls of this study (North Male, Ari and Baa) are currently the most developed for tourism and account for over 80% of tourist diver revenue and include 63% of regularly visited manta ray watching sites. Annual tourist numbers in the Maldives have grown consistently over the past decade except for 2005, following the December 2004 tsunami, reaching 680 000 entries in 2008. The increased intensity is expected to have some impact on the marine environment and there is awareness of the environmental issues and the need for sustainability within the tourism industry (Ministry of Tourism and Civil Aviation 2007). Although a major diving industry has developed in the Maldives over the last few decades (at the end of 2008 there were 94 resorts with a dive base and 140 registered liveaboard vessels) which is partly reliant on these animals, no scientific study has been published on the size of the Maldivian *M. alfredi* populations to date. The presence of manta rays is often unpredictable and since the size of their population is unknown, it is difficult to predict how the extensive tourism pressure will affect their normal behaviour. In order to keep the tourism business sustainable and to prevent extensive disturbance of manta ray populations it is important to estimate the size of the present population around the atolls and main tourist dive points, as well as to follow its changes.

The population being estimated is that of manta rays which visit cleaning stations in North Male, Ari and Baa atolls. This excludes juveniles (<2 m disc width), which are rarely seen at cleaning stations. Anderson et al. (2011) describe the migration of manta rays in the Maldives with seasonally reversing monsoon currents with manta rays occurring on the leeward side of atolls to the prevailing monsoon winds. Kitchen-Wheeler (2008) investigated manta movements in the Maldives and showed that individual manta rays move between east and west sides of atolls with the monsoon seasons and mantas in North Male atoll alternate between cleaning stations on either side of the atolls depending on season. In North Male and Ari atolls, manta rays were also reported at different cleaning stations on the leeside of the same atoll during a single season. There was a low level of movement between atolls detected (<1% of individuals); this was considered insignificant for the purposes of this study in the context of an atoll population being considered closed or open. Thus manta rays sighted from any cleaning station within an atoll were assumed as coming from a single atoll population. It was decided to try to estimate atoll populations rather than site populations due to the known movement of mantas among sites within atolls and the expectation that due to minimal movement between atolls, that a population might be considered limited to a geographical atoll.

Since it is unknown how far manta rays migrate around the atolls and whether there are resident populations or not, closed (Petersen 1896) and open

(Seber 1982) population models were used to cover both possibilities. Petersen (1896) pioneered estimating population parameters using mark-recapture techniques and many studies have employed and developed these methods (Cormack 1968; Otis et al. 1978; Cormack 1979; Pollock 1982; Seber 1986; Schwarz and Arnason 1996). Petersen's method remains one of the most popular models for estimating the size of a closed population. The model requires that (1) there is no immigration or emigration; (2) all animals are of the same likelihood to be captured; (3) marks are not overlooked or lost/missed by observers and (4) the catchable population is the total population, while any portion of the population that is not subject to collection is not included in the estimate (Gatz and Loar 1988; Pollock 2000). It was expected that the atoll population might be considered closed as deletions from deaths or permanent emigration and additions from young adults were expected to be low or negligible over the timescales of these studies and the known lifespan of *M. alfredi* (35+ years). Marshall and Bennett (2010a) report a significant proportion of manta rays in southern Mozambique bearing scars from shark attacks. The vast majority of individuals in the Maldives are unscarred. Marshall and Bennett (2010b) showed manta rays surviving even from several large bites. It is likely that manta rays are not attacked as frequently by sharks in the Maldives, as in Mozambique. Therefore, in this study, the losses from shark attacks were considered insignificant. It is probable that there are cleaning stations currently unknown to us which are visited by a number of manta rays. Some animals might thus be missed in atoll population estimates. Using photo identification instead of real capture greatly reduces the negative effect of trap response on the animals, therefore this limitation can be considered as not significant. The most serious limitation of this method is that different animals may have inherent variability in being sighted at survey sites. Factors which may affect capture probability include individual heterogeneity (preferences due to sex, size, age, shyness and boldness of individual animals) and temporary emigration. When animals exhibit heterogeneous capture probabilities, any available estimate of population size is likely to be markedly biased (Cormack 1968). The CAPTURE (open) model developed by Otis et al. (1978) which allows capture probability to vary with individual animal requires that large numbers of animals are caught in each sample and animals are caught a large number of times. It was known when the experiments were devised that (1) the majority of manta rays were not re-sighted, and (2) there were frequently long period between sightings thus the commonly used models might not be useful. The open model encompasses additions (recruits and immigrants) and deletions (deaths and emigrants) and is useful when studies are performed over longer period of time (years). The open model was developed independently by Jolly (1965) and Seber (1965) and requires equal catchability and equal survival rates for all animals within each sampling time and this had to be assumed despite anticipated individual heterogeneity. The maximum time between samples used for the Jolly-Seber model study was 13 months (Lankan Reef) and samples were taken in a single day, so relatively instantaneous. Since it is so difficult to know if assumptions are satisfied or not, both open and closed models were used to see which gave the most sensible results.

The use of mark-recapture tools have long been used in studies of fish population and stock assessment (Hoening et al. 1998), but the technique has rarely been applied in elasmobranch species (Kohler and Turner 2001). During this study sight-resight, instead of mark-resight, a variation of mark-recapture technique was applied. This involved the positive identification and subsequent re-sighting of identified individuals based on their natural markings (distinctive marks, scars, and pigmentation), a widely used method which has been applied to a vast array of organisms and is much less disruptive to the animals than tagging. Using natural markings for identifying individuals is common in large marine species and this technique has been used for population estimates of nurse sharks *Ginglymostoma cirratum* (Castro and Rosa 2005), zebra sharks *Stegostoma fasciatum* (Dudgeon et al. 2008), bottlenose dolphins *Tursiops truncatus* (Silva et al. 2009) and humpback whales *Megaptera novaeangliae* (Blackmer et al. 2000). Individuals of each manta ray species may also be identified from physical characteristics and patterns of ventral markings that are unlikely to change over time (Marshall et al. 2009; Kitchen-Wheeler 2010). A summary of variations in physical characteristics and distinctive ventral markings of each species and that can be used to identify individuals is shown in Fig. 1.

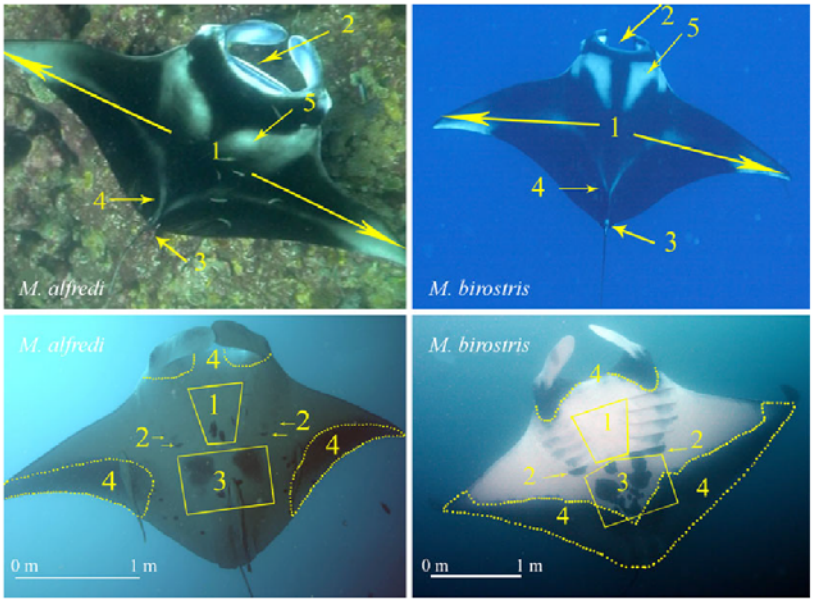

Fig. 1 Physical characteristics, markings and pigmentation patterns to differentiate between individuals and species of *Manta* (Marshall et al. 2009; Kitchen-Wheeler 2010). *M. alfredi* (top left) can be differentiated from *M. birostris* (top right) by: (1) its smaller size (max disc width 5 m vs. 7 m); (2) top jaw lacks enlarged denticles; (3) the absence of a postdorsal protruberance with spine; (4) both species have chevron and black colour types, the chevron is annotated; (5) anterior edge of shoulder patches runs medially from the spiracle in *M. birostris* and shape of markings are less curved and more geometric than *M. alfredi*. Individuals of species can be identified by ventral markings (bottom photos). (1) Ovoid markings between gill slits are the primary identifier for individuals of *M. alfredi* but are always absent in *M. birostris*. Markings at distal ends of gill slits (2) can be used as an objective differentiator and recorded as absent or present for each gill slit. Markings in lower abdominal area (3) are individually distinctive and different types of marks are present depending on species. Pigmentation around the head and posterior-ventral part of fins (4) varies between individuals and can be diagnostic of species

From long term studies of *M. alfredi* in Japan we know that individuals' markings remain constant over a period of over 20 years (Homma et al. 1999). The identification method used for these surveys has shown that the ventral pigmentation of individual manta rays was consistent during the 9-year study period (Fig. 2) (Kitchen-Wheeler 2010).

There are reports of manta rays being sighted from all atolls in the Maldives (Anderson et al. 2011). The surveys were conducted in three of the 26 geographical atolls and the results from the surveyed atolls were then used as representative of small and large-sized atolls to estimate the total population size of *M. alfredi* for the entire Maldives.

Methodology

Study sites

In order to minimise bias in population estimates caused by small samples, surveys used for population estimation were conducted at times when manta ray numbers were likely to be at their highest, based on previous experience at the sites. The study sites were well known fish cleaning stations in North Male atoll (Lankan Reef and Boduhithi), Ari atoll (Table Thila and Madivaru) and Baa atoll (Nelivaru, Dhigu Thila, Donfanu Thila), that are regularly visited by manta rays during the appropriate monsoon period. In

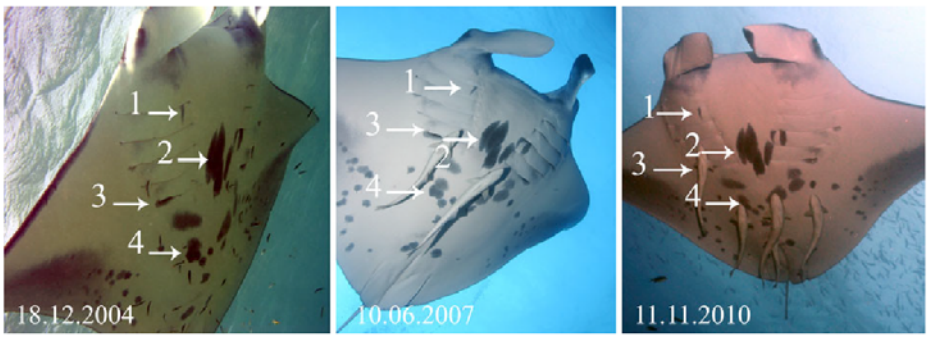

Fig. 2 Ventral pigmentation and markings vary insignificantly with time. This sequence shows how four markings sets: (1) a small vertical line located under first right gill slit; (2) pattern of large ovals located between the gill slits; (3) pigmented end of fifth right gill slit and (4) pattern of large circular mottles in lower abdominal area) appear the same over a period of six years

addition, Hanifaru lagoon (Baa atoll) which is also an important feeding area for manta rays and includes cleaning stations (Figs. 3 and 4). Each atoll has an administrative name and a geographical name (in italics in Fig. 4) and either one are in common use. In this study, South Maalhosmadulu will be referred to as Baa atoll.

Cleaning stations are ideal to make such observations, since manta rays often visit these places in order to be cleaned of parasites and dead skin (Gooding 1964; Feder 1966; Youngbluth 1968; O'Shea et al. 2010). Manta rays tend to move slowly around cleaning stations and are therefore easier to approach and photograph. Feeding areas also proved to be very efficient data collection sites because of the high number of individuals, but these locations are less predictable for the presence of manta rays and fast movement of animals may make data collection

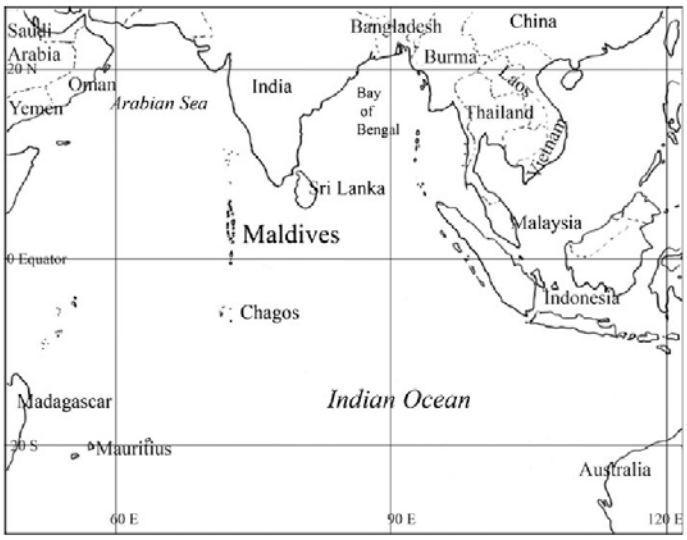

Fig. 3 Indian Ocean to show Maldives, located centrally to south-west of India

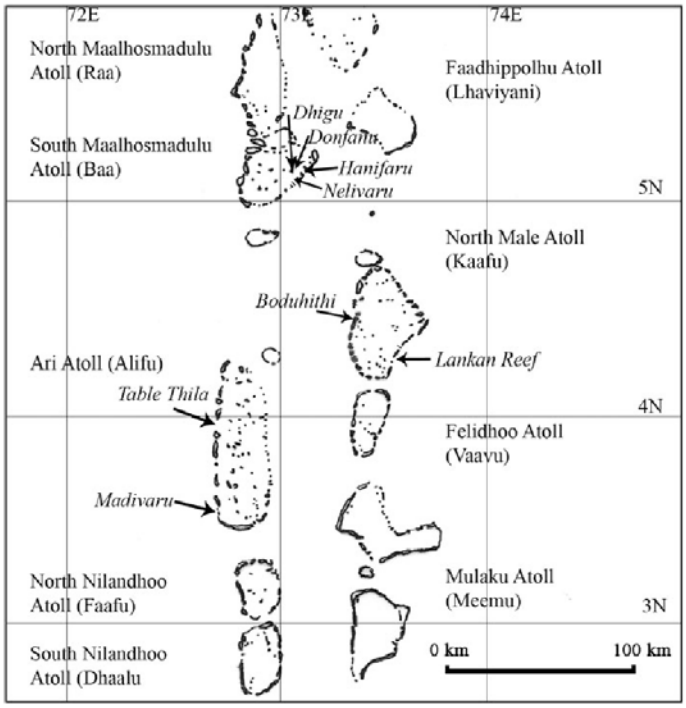

Fig. 4 Central Maldives atolls, including Baa, North Male and Ari atolls. Each atoll has geographic and administrative (in brackets) names and have alternate common use. Survey sites are arrowed

unmanageable. The survey sites in this study vary in size and topography. Lankan Reef, Boduhithi and Madivaru are located on sections of the outer fringing reef. Lankan Reef cleaning station is a large bommie, approximately 8 m×8 m, at 15–22 m depth on the reef slope. Boduhithi is an area of reef of approximately 100 m×50 m at around 3 m depth on the reef flat. Madivaru consists of a number of small cleaning stations along the crest of the outer reef at around 10 m depth, extending approximately 300 m into a channel. Table, Nelivaru, Dhigu and Donfanu are submerged reefs or *thilas* (a Maldivian word) and the cleaning stations are specific areas of the *thilas*. Hanifaru site is the south-eastern section of the faro or ring reef of the same name. At Hanifaru, the reef structure creates a secondary lagoon which traps outflowing plankton and includes some coral bommies which are cleaning stations. For the purpose of this study, the objective was to capture the maximum possible number of animals observed during each observation period (45–60 min).

Data collection

Two types of surveys were undertaken: routine and intensive (n=329 and n=100 respectively). The routine surveys were conducted from December 2000 to August 2009; these were primarily during tourist diver visits to cleaning stations. Each involved a 45–60 min scuba dive during which manta rays were individually identified using the methods described in Kitchen-Wheeler (2010). These surveys identified the majority of the animals visiting each site and were conducted when encounters with manta rays were though likely, based on experience. Approximately two surveys were made per week in the relevant season but the rate of survey varied from one year to the next.

In addition, in 2006 (North Male atoll), 2007 (Ari and North Male atolls) and 2009 (Baa atoll), one hundred intensive surveys were conducted during 23 full-day studies conducted at cleaning stations plus

Hanifaru feeding area; these were undertaken primarily to estimate population size. Survey dates for these studies were selected with the aim of obtaining as large a sample as possible on each day (the greater the number of animals identified the larger the number of re-sighted manta rays likely) based on experience of the site and conditions. The time spent underwater during the intensive full-day studies varied from 2 to 10.5 h per day (comprising from two to fourteen 45–60 min surveys), depending on conditions and manta ray activity. At Lankan Reef, Boduhithi and Madivaru the sites were surveyed continuously for 6–10.5 h. At other sites surveys were only commenced if manta rays were confirmed to be present during a reconnaissance by a snorkeler. Two to six observers were deployed per survey. Divers either waited nearby the cleaning station throughout the period (Lankan Reef, Table thila sites) or made transects of the reef to visit the different potential cleaning stations or feeding areas (other sites). Surveys from the intensive full-day studies were combined to calculate population estimates using the method of Petersen (1896) and Jolly-Seber (Seber 1982,1986). Full day samples were used in all the population estimates to standardise the samples and so they might be considered comparative for effort.

The annual monsoon cycle affects manta movements, therefore manta rays may be predicted to visit feeding and cleaning areas located on the east and west of atolls in the lee of the prevailing monsoon wind where productivity is high due to phytoplankton blooms (Anderson et al. 2011). Data were collected in both monsoon seasons in North Male atoll, this being the only atoll with regularly visited manta ray cleaning stations on both sides (the other atolls have cleaning stations on both sides but sightings on one side are much less reliable and so such stations are rarely visited). It had already been established that many manta rays which visited Lankan Reef also visited Boduhithi cleaning stations during the other monsoon and therefore came from the same population (Kitchen-Wheeler 2008). Intensive surveys in North Male atoll were undertaken at Lankan Reef in July 2006 and July and August 2007 and at Boduhithi on three days during the NE Monsoon season of 2007, in between the Lankan surveys. Thus the North Male intensive surveys were carried out over a period of around 13 months. The surveys in Ari atoll were conducted in February and March 2007, approximately a month apart. The surveys in Baa atoll were made on consecutive days from the 3rd to 18th August 2009, but no survey was possible on 14th and 15th for operational reasons. These surveys were made at Dhigu, Donfanu, Nelivaru and Hanifaru sites with the majority of time spent at the latter site. As the sites were in close proximity (<5 km apart except Nelivaru which was approx 10 km distant) and on several occasions manta rays would be first sighted at a cleaning station in the morning survey (including Nelivaru) and then be seen feeding at Hanifaru during the afternoon surveys, observations from full-day studies at the various east Baa atoll sites were combined.

Individual manta rays of both species (*M. alfredi* and *M. birostris*) can be identified by the pattern of ovals, spots and mottles on the ventral side (Figs. 1 and 2) (Kitchen-Wheeler 2010). Ventral marking patterns, along with sex, size and other important physical characteristics of individuals were recorded on a combination of pre-prepared underwater slates, digital video and still cameras images. These were reviewed after each dive to identify which manta rays were present by comparing them to the ventral markings of animals already identified and included in an Access database (Kitchen-Wheeler 2010). Size was estimated by comparison to nearby divers and was recorded as part of the identification process so that a range of sizes could be shown in the samples. Sexual maturity can be assumed in females if they are clearly pregnant. Pregnancy was determined from obviously distended ventral and dorsal surfaces. Marshall and Bennett (2010b) also considered females with mating marks to be mature, however this characteristic was not routinely recorded in this study until after 2008 so data are missing from the majority of female records. Males are harder to identify as being sexually mature but their status can be determined by the state of the claspers. A mature male ready to mate has enlarged and calcified claspers (Pratt and Carrier 2005) or claspers extending beyond the posterior edge of the pelvic fins, following Walker (2005) and White et al. (2006). These features can easily be determined from visual assessment of the claspers and genital area either during observation, or from analysis of photographs. Males with partially extending claspers, lacking calcification were considered sub-adult and males with partially formed

claspers or claspers not extending beyond the pelvic fin were considered juvenile as in Marshall and Bennett (2010b). All database photographs of males were assessed to investigate the state of claspers and determine whether mature, sub-adult, juvenile or unknown (genitalia missing from database photograph or unclear). Sometimes not all animals present could be identified for a number of reasons including insufficient data to confirm identification or the manta ray did not approach the observer closely enough for appropriate data collection.

Estimating population size

Both the closed population model (Petersen 1896) and open population model of Jolly-Seber, (Seber 1982, 1986) were used to estimate population size in this study as it was unknown which model was appropriate. The Cormack-Seber-Jolly model was not used as the time varied substantially between the intensive samples (used in this study) or the intensity of surveys varied considerably between sites and year to year if all the database samples were to be pooled by year (Cormack 1964; Jolly 1965; Seber 1965). Full-day intensive studies were used to standardise the samples. The populations were assumed closed in the Petersen model methods although a closure test was not performed as it was expected that variations in capture probabilities caused by behaviour of this species would be difficult to distinguish from failure of closure (Otis et al. 1978). To estimate the size of the manta ray population, probabilistic estimators were employed based on the probability that an animal captured on a first survey would be captured on a second survey. Once an animal was individually identified it was considered 'marked' and 'recapture' was based on re-sighting 'marked' individuals.

Two approaches were used to estimate populations using Petersen's method (closed model). In the first, 'marked' individuals consisted of all the animals which had previously been identified in that atoll (in both routine and intensive surveys) at any time earlier than the date when the population was estimated (method 1). In the second, two full-days of intensive surveys were compared with the marked population identified only from the earlier intensive survey day (method 2). The two days were up to ~13 months apart. The period of sightings data used in method (1) varied depending on the days when the intensive full-day studies were carried out: for Lankan reef (North Male atoll) this involved manta rays sighted between November 2001 and August 2007; for Boduhithi (North Male atoll) sightings between November 2001 and March 2007; for Ari atoll sightings between January 2002 and March 2007; and for Baa atoll between October 2007 and August 2009. The majority of sightings from North Male and Ari were obtained from 2003 onwards (see Fig. 6) so most of the data used to provide the marked sample in the Petersen (1) method for these atolls was obtained over 3.5–4.5 years. The Jolly –Seber analysis only used samples from the 23 full-day studies with all surveys from each day pooled thus sightings were a maximum of 13 months apart.

The largest possible samples were required to reduce error as small samples often produced zero re-sightings which cannot be used for population estimation. A days worth of surveys were pooled in order to create a large sample (Bailey 1951). Other researchers of manta ray populations have pooled a number of surveys over periods of months and then performed analysis comparing "year" samples (Deakos et al. 2011; Marshall et al. 2011). This method was unnecessary because a day sample contained similar numbers of animals sighted in a season in the other areas and made the sampling periods relatively instantaneous. Two different marked samples methods were used with the Petersen model as it was hoped that using the large, 'all the animals which had previously been identified in that atoll' as the marked sample would provide the biggest possible sample of marked animals and avoid the errors in small samples, but this method would likely violate the assumptions of a closed population. Method (2) involved the comparison of discrete samples within a much closer time-frame so less likely to compromise the closed population assumptions. In all three methods, samplings were repeated where possible so that means could be calculated (Cliff et al. 1996; Castro and Rosa 2005).

In summary: the benefit of method (1) was that the known marked sample was high (all database entries for the atoll) with the sampling surveys conducted on single days several times for each atoll over an extended period of time (several weeks, up to 13 months). The issue with this method was that the sampling period for the North Male and Ari samples was greater than 3 years so likely to violate the closed

assumption. The benefit of method (2) was that the sample periods being compared were the same and the maximum period of 13 months minimises the likelihood of emigration, deaths and immigration and thus the population could be considered closed. The model has been applied in this study as in Matthews et al. (2001), without the modification proposed by Bailey (1951) as the known marked population and sample sizes were relatively high. The Jolly-Seber estimator was used to analyse the Lankan, Boduhithi (separately and combined) and Baa full-day intensive study data. It could not be applied to the Ari atoll data as only two full-day studies were undertaken, and there were no animals re-sighted on the second date. Survival probability and re-capture probability were assumed to be constant over the time of the study.

Other analyses

Discovery curves for the three atolls were created. They show the total number of individual manta rays identified at cleaning stations and feeding areas within each atoll as a function of the number of 45–60 min surveys undertaken. As the number of identified individuals approaches the population of an atoll the discovery curve is expected to reach an asymptote. If no asymptotic trend is detected this suggests that a significant proportion of the population is yet to be identified.

The *M. alfredi* atoll population size was extrapolated using the results of the surveyed atolls as representative small and large-sized atolls to give an estimated population for the whole Maldives. The 26 atolls were grouped geographically to create 'natural' "atoll areas" which typically consisted of a main atoll plus nearby minor atolls and atoll islands (where applicable) e.g. Ari atoll consists of the main Ari atoll plus the much smaller neighbouring Rasdhoo atoll (Fig. 4 unmarked, located to NE of Ari atoll). This geographical grouping produced 17 atoll areas. When referring to an atoll in this document hereafter, an atoll area is being discussed. The areas of each of these 17 atoll areas were compared; two were significantly larger than the other atolls and were considered large atolls (Huvadhoo and Ari). It was assumed that population is related to an atoll and that the population size is likely to be related to the size of the atoll. In this study two small atoll populations (North Male and Baa) and one large atoll (Ari) were investigated. Population estimates for the studied atolls were considered representative of small and large atolls and multiplied by the number of small and large atolls to estimate a population for the Maldives.

Results

Sightings data (3373 sightings) of 1,835 individual manta rays (range 1–35 sightings per animal) in the Maldives were analysed. 97.3% of animals were observed in the three atolls investigated in this report. The remainder were seen in nine other less frequently visited atolls. Seven *M. birostris* were recorded in the database but none were identified on any of the intensive all-day survey dates, nor has been re-sighted to date. Only 17 out of 1835 individuals (<1%) were resighted in different atolls to that in which they were initially sighted. Thus movement between atolls was considered negligible for the purposes of this study. Overall only 454 (24.7%) animals were re-sighted (North Male: 34.5%, Ari: 17.2%, Baa: 17.6%). The longest gap between re-sightings was 7 years 11 months, with 7 other animals having a gap in sightings of over 5 years and a gap of >3 years was found to be relatively common (>100 examples). Each year approximately 200 new manta rays have been positively identified (Fig. 9) and added to the database which now contains the largest number of identified manta rays for any country in the world.

For manta rays with recorded estimated size in the three studied atolls, the mean estimated disc width (DW) of females was 3.26 m (SD±0.513, range: 1.75–4.50 m, n=870), and that of males was 2.69 m (SD±0.407, range: 1.75–4.0 m, n=504). Females were significantly larger than males (2-sample t-test: t=−22.89, p<0.001). Overall, the population shows a strong sex bias with 35.7% males vs. 64.3% females in the known 1,835 animals (ratio 1:1.8) (χ^2=149.06, df=1, p<0.001). The gender mix at the survey sites in this study are reported in Fig. 5. These results suggest that both males and females of size ≥1.75 m DW visit the cleaning stations and feeding areas included in this study so the population being measured shows a broad range in sizes.

Only 112 females were ever observed pregnant and thus assumed mature. The mean estimated DW of known mature females was 3.73 m (SD±0.354, range 3.0– 4.5 m). Pregnant females were significantly

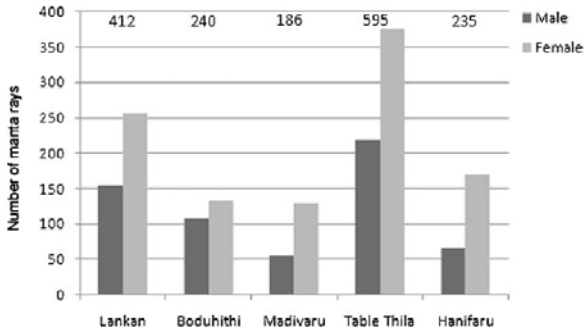

Fig. 5 Gender distribution of identified manta rays at study sites during the entire study period. Numbers across the top indicate total number of individuals observed per site

larger than all females (2-sample t-test: $t=12.47$, $p<0.001$). Of photographs of 198 males which could be evaluated, 193 (>97%) were assessed as mature. The mean estimated DW of adult males was 2.81 m (SD± 0.380). Of the other five, four were assessed as sub-adult and one as juvenile. The apparently high proportion of mature males in this sample with photographed genitalia may be due to larger males being less intimidated by photographers, allowing larger number of photographs to be taken including the genitalia. Photographs of other males were available but the genitalia were either missing from the photograph could not be assessed with confidence.

Only 3 small juveniles were included (<2 m DW). Small (<2 m DW) manta rays are relatively commonly seen inside large lagoons where boats anchor but were rarely reported at the cleaning and feeding areas studied, thus very young mantas are not included in the population estimate.

Sightings data for North Male atoll included data for 709 identified manta rays of which only 254 (35.8%) were re-sighted (1–34 times) during the entire study. Of animals seen at least twice, 105 were sighted on only east side sites (range 2–16 times), 23 were only seen on the west side sites (range 2–4 times) and 126 were sighted on both sides (49.7%). Being sighted on both sides of atolls showed a strong correlation with increased number of sightings (Pearson correlation = 0.718, $p=0.001$) and of manta rays sighted "frequently" (≥6 times) 79.2% were sighted on both sides of the atoll. Given this, it is assumed that there is a single population of manta rays which use both Lankan Reef and Boduhithi and thus the estimates of populations from surveys at both sites are of the same population i.e. that of North Male atoll.

The mean number of manta rays seen per survey at Boduhithi was 13.7 (SE mean=1.63, $n=47$) and was significantly more (2-sample t-test: $t=3.86$, df= 55, $p<0.001$) than the 7.1 per survey (SE mean=0.51, $n=177$) seen at Lankan.

The first population estimate was calculated using the known (marked) population for the entire atoll up to the date of the survey and calculated using the Petersen's method (1). Figure 6 shows the proportion of marked animals in each sample using this method. The proportion of marked animals in each sample varied from 4.3% (east Baa sites on 5.8.2009) to 88.8% (Lankan Reef on 8.8.2007). Based on this model the mean estimate for the population of *M. alfredi* in North Male atoll was 716 (SD±69.9) based on survey data from Lankan Reef, and 1441 (SD± 465) based on survey data from Boduhithi. The mean estimates for the atoll population derived from Lankan Reef and Boduhithi studies were compared using a one-way ANOVA-test. The estimates are significantly different ($F=10.07$, $p=0.025$), nevertheless they were combined as they are both estimates of a North Male population; mean 1026 (SD±474) manta rays. The mean estimated population for Ari atoll was 1468 (SD±634), and for Baa atoll was 719 (SD± 631) animals which is not significantly different to combined North Male result (one-way ANOVA: $F=1.24$, $p=0.281$). Summarised results of population estimates using method (1) are shown in Table 1. The ranges of results using each data set are indicated in Fig. 7.

The estimates using Petersen's method (2), when two full-day studies of intensive surveys were compared with the 'marked' population which included identified individuals only from the first

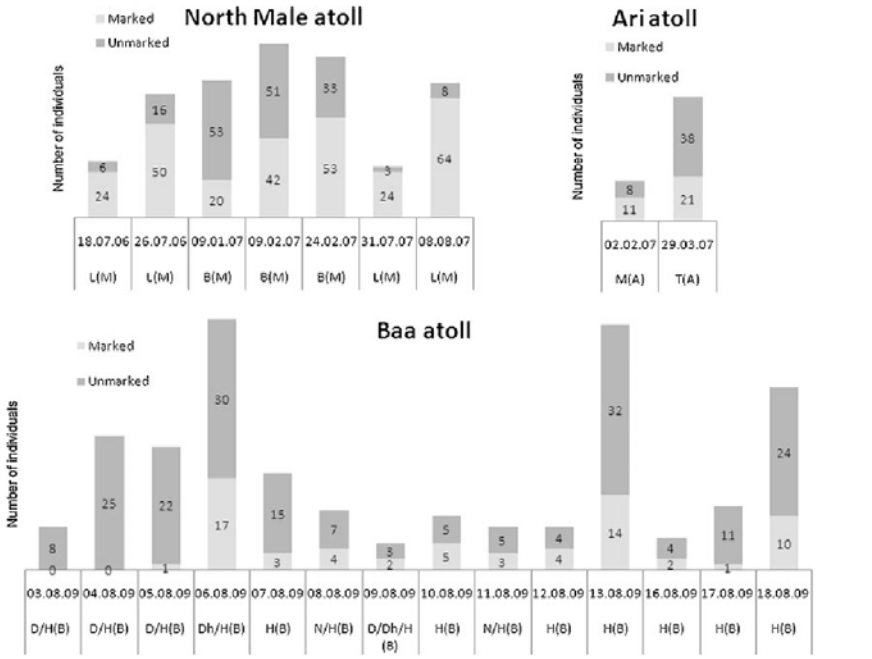

Fig. 6 Number of "marked" and "unmarked" manta rays per sample used in Petersen's method (1) analysis. "Marked" population being individuals previously seen before in the atoll at any time prior to the survey date. (L: Lankan; B: Boduhithi; T: Table Thila; Ma: Madivaru; Dh: Dhigu Thila; D: Donfanu; N: Nelivaru; H: Hanifaru)

Table 1 Population size estimates (N) assessed for atolls and sites by different estimation methods

Model of population estimation	Atoll	Mean Population estimate (N)	Range of estimates
Petersen's method (1) (entire atoll as marked sample)	(Lankan) North Male	716 (SD±68.9)	633-774
	(Boduhithi) North Male	1441 (SD±465)	1051–1956
	(Combined) North Male	1026 (SD±474)	633-1956
	Ari	1468 (SD±634)	1019-1916
	Baa	719 (SD±631)	298-2388
Petersen's method (2) (paired samples, earlier day as marked sample)	(Lankan)North Male	330 (SD±243)	139-810
	(Boduhithi) North Male	563 (SD±159)	381–990
	(Combined) North Male	536 (SD±244)	139-1222
	Baa	301 (SD±183)	68-850
Jolly-Seber	(Lankan) North Male	181 (SD±70.7)	131-231
	(Boduhithi) North Male	371 (only one result)	371 (only one result)
	(Combined) North Male	538 (SD±422)	139–1172
	Baa	204 (SD±163)	60-588

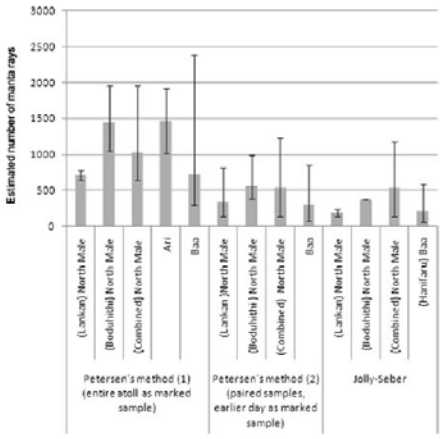

Fig. 7 Mean and range of population size estimates assessed for sites (Lankan Reef and Boduhithi) and atolls by different estimation methods

sampling day. This method could only been used for North Male and Baa atolls because no manta rays were seen on both of the full day sampling studies in Ari atoll. For Baa, only population estimates from full-day surveys when at least 10 animals were captured and at least one animal was matched on the paired dates were analysed. Small samples will cause a negative bias in estimates (Otis et al. 1978). Based on this method the mean atoll population estimates were 330 (SD±243) for North Male atoll population based on Lankan Reef data; 563 (SD±159) for North Male atoll population based on Boduhithi data; 536 (SD±244) for North Male based on combined Lankan Reef and Boduhithi data (n=21 paired results, range 139–990) and 301 (SD±183) for Baa based on East Baa atoll sites data (n=23 paired results, range 68–850). The estimated mean population for North Male atoll using the combined sites is 536 (95% CI 425–647). When the estimates of atoll population using Petersen's methods (1) and (2) were compared using one-way ANOVA the estimates for all sets of data were statistically different (Lankan Reef: F=9.24, p=0.016; Boduhithi: F=9.56, p=0.037; North Male combined: F=12.97, p =0.001; Baa: F=8.91, p=0.005). Thus, population estimates using Petersen's method (2) were significantly smaller than estimates using method (1).

Using the Jolly-Seber method applied to the full-day studies of intensive surveys for North Male and Baa sites, the following population size (N) was estimated (summarised in Table 1 and Fig. 7): mean 181 (SD±70.7) individuals based on Lankan Reef data and 371 for Boduhithi data (single result). Using combined Lankan and Boduhithi samples (n=7) with the analysis performed on the samples in chronological order, the population estimate was 538 (SD±422). These samples were obtained over approximately one year so this result suggests that the upper estimate of population in North Male atoll in a year is 1172 using the Jolly-Seber method. Mean population estimates for both sites individually and combined using Petersen's model method (2) and Jolly-Seber were not significantly different (Lankan data: F=0.67, p=0.446; Boduhithi data: F =1.08, p=0.407; combined: F=0.00, p= 0.984). For Baa atoll sites using all fourteen intensive full-day survey results the mean estimate using the Jolly-Seber method was 204 (SD±163) and was not significantly different (F=2.24, p>0.14) to that obtained using the Petersen method (2).

Discovery curves of identified population for each atoll are shown in Fig. 8. The number of new manta rays discovered varies substantially between surveys (range 1–38). For example, in North Male atoll, a single survey in July 2006 identified 17 new animals, but the next day when 10 consecutive one-hour surveys were carried out, a maximum of four new animals were discovered per survey, causing the curve to flatten out again. The sequence of surveys 161–163 (January 2007) in the North Male curve were for surveys at Boduhithi and 10, 17 and 12 new animals were identified (respectively). Similar spurts in numbers of new animals are observed around surveys 128–130 (March 2007) at Table thila in Ari atoll, identifying 18, 38, 22 new animals and in the Baa curve when 21 and 33 new animals were recorded during surveys 11 and 24 (August 2009) respectively. There is no explanation for this phenomenon; these high results are recorded from all sites and at different times of year. Figure 9 reports all manta ray sightings reported with time. In North Male atoll, manta rays are reported year round; whilst in Ari they are infrequently reported during the SW monsoon (un-shaded area). The rate of growth is hindered during periods when surveys were not (or very infrequently) conducted e.g. April-November 2008. The discovery curve for North Male appears to be

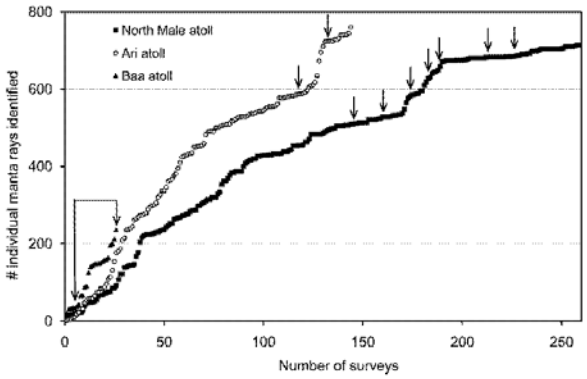

Fig. 8 Discovery curves for new individuals identified in North Male, Ari and Baa atolls. New manta rays are reported per survey in each atoll, in chronological order. Only the North Male curve shows an asymptote indicating that most manta rays have been identified. Start of intensive surveys used for population estimates for North Male and Ari atolls annotated with arrows. Bracketed arrows show period of intensive surveys for population estimates in Baa atoll

reaching an asymptote (Fig. 8) so the rate of discovery of new animals is levelling off suggesting that the majority of the atoll population have been identified that the atoll population is around the 700+ manta rays already identified. The regressions for Ari atoll and Baa atoll were linear suggesting the atoll populations exceed the 769 and 231 manta rays currently known from these atolls, respectively.

The low level of inter-atoll movement (<1% manta rays were reported from more than one atoll) suggests that individual *M. alfredi* typically visit cleaning stations in a single atoll and thus populations should be atoll specific. Assuming the restriction to a single atoll being probably caused by geography (the move to a different atoll would require a traverse of deep ocean) we may assume that *M. alfredi* are limited to the atoll areas as defined in the method, and that as manta rays have been reported from all atolls, each atoll will have a population of manta rays. The northern group of atolls include some joined sections but were treated as three small atoll areas based on the area and shape of this region. The two southern atolls (Addu and Foamullah) were treated as a single small atoll for the purposes of this exercise as the areas of each individually is very small yet Addu atoll alone is expected to have a population of several hundreds of manta rays based on recent unpublished studies by the authors. This geographical grouping creates seventeen "atoll areas" of mean area 1381 km² (SD±811 km²). The atolls of Ari (2,770 km²) and Huvadhoo (3285 km²), when compared to the mean area of the other atolls using one-way ANOVA are significantly

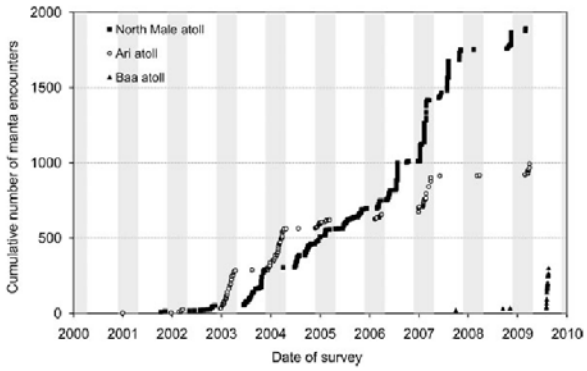

Fig. 9 Cumulative number of all manta rays sighted in North Male, Ari and Baa atolls with date, illustrating rise in total number of sightings over study period. This graph illustrates study period and intensity of surveys in each atoll with time. Shaded area is period of NW monsoon and unshaded area is period of SW monsoon each year

larger (F=20.97, p<0.001) and are thus considered large atolls, and the remaining 15 are small atolls. The 15 small atolls do vary in area quite considerably (range: 208–2026 km²) but all are known to have populations of manta rays. As the two atolls of Ari and Huvadhoo are geographically larger than the other atolls they would be expected to maintain larger populations of manta rays.

The estimates of atoll population for North Male using Petersen's method (2) and Jolly-Seber are very similar (F=0.00, p=0.984) and the results can be used as a baseline for an estimated small atoll population i.e. ~537 animals. The Baa estimates are significantly lower than the combined North Male estimates but are similar to the results from Lankan. That both results are obtained from sites on the east side of atolls during the SW monsoon makes them even more comparable and suggests that the Baa estimates are more likely to be those of the population around a site than of an atoll. Unfortunately we were unable to obtain a Petersen's (2) or Jolly-Seber estimate for a large atoll. The Petersen's (1) estimate for Ari atoll was just over 50% greater than the North Male atoll estimate obtained by the same method (1468/1026= 1.51). By proportion we might estimate the population for a large atoll to be 537×1.51=811 manta rays. This figure seems reasonable based on the discovery curve indicating the population for Ari atoll is over 769 known animals. Using the estimate of 537 for the 15 small atolls and 811 for the two large atolls suggests that the Maldives *M. alfredi* population is around 9,677 rays.

Discussion

Three different methods, with different assumptions were used to obtain population estimates. The Petersen (1) method produced the highest estimates however the assumption of closure was likely to be violated for the North Male and Ari atoll estimates due to the length of time over which the data for the 'marked' samples were gathered and thus the estimates are probably overstating the populations. The estimates obtained from Petersen (2) and Jolly-Seber methods were similar for both North Male and Baa atolls. It is likely that the estimates using these methods for North Male atoll, which were obtained using samples from both sides of the atoll and obtained over a 13 month period do reflect the annual population of the atoll. Castro and Rosa (2005) estimated the abundance of nurse sharks (*Ginglymostoma cirratum*) at Atol das Rocas off north-east Brazil using Petersen-Bailey and Jolly-Seber models and also obtained statistically similar results using both methods.

The strong bias towards females in the population visiting the cleaning stations in the Maldives (64.3%) was less (Yates corrected χ^2=19.99, df=1, p<0.001) than seen in Mozambique where Marshall and Bennett (2010b) found approximately 75% of the *Manta alfredi* population was female. This is in contrast to the population in Maui, Hawaii where only 46% were females (Deakos 2010). This result was significantly lower than the Maldives result (Yates corrected χ^2=32.99, df=1, p<0.001). The site with the highest proportion of females was Madivaru (2.7:1) which was similar to Mozambique (Yates corrected χ^2=0.147, df=1, p=0.702), whilst the lowest proportion of females was observed at Boduhithi (1.3:1) but this was still significantly higher than Maui (Yates corrected χ^2=4.309, df=1, p=0.038). Sexual segregation in other elasmobranch species, e.g. scalloped hammerheads *Sphyrna lewini* and sandbar shark *Carcharhinus plumbeus*, is postulated to maximise breeding potential (Springer 1960; Klimley 1987). However, sexual biases occur in many species of elasmobranchs, varying with location and time and it is presently unknown why there is so much variation in *M. alfredi* sex ratios (Anderson and Pyle 2003; Castro and Rosa 2005; Graham and Roberts 2007; Marshall et al. 2011).

North Male atoll

The difference in population estimates obtained from surveys at Lankan Reef and Boduhithi individually using all three methods may be due to differences in the sizes of the cleaning stations. Lankan is a large bommie with approximately four areas of cleaners which can accommodate one manta ray each, so a maximum of four animals may clean simultaneously (although up to 20 other manta rays may assemble around the cleaning station and wait for a cleaning station to become free). Boduhithi is a reef flat with dozens of cleaner fish areas and may accommodate 40+ animals cleaning simultaneously. Lankan Reef has a higher proportion of visiting females compared to Boduhithi (62.4% vs. 55.4% (Yates corrected χ^2=

4.377, df=1, p=0.036)) and less mantas are seen at Lankan per survey than at Boduhithi. The mean size of female manta rays is significantly larger than male manta rays generally. Observations at Lankan Reef cleaning station report a higher proportion of (larger) females. This may be because (smaller) males may face competition for access to the cleaning station. Studies in other elasmobranchs have shown sharks display subordinate behaviour to individuals which are larger (Myrberg and Gruber 1974; Klimley 1983). We propose that competition may be the cause of the differences in population estimates and gender mix of manta rays seen at Lankan compared with Boduhithi. At Boduhithi there are many areas of cleaner fishes, allowing more manta rays to be cleaned simultaneously so smaller animals face less competition for cleaning station access and a higher proportion of the population (in particular smaller/male animals) will be reported, resulting in the population estimates being greater for Boduhithi. Despite this, very few juveniles are reported at cleaning and feeding areas although they are occasionally seen but not positively identified, in shallow lagoons within the atolls. The population estimates will miss these juveniles and any individuals that do not visit the cleaning and feeding areas investigated and so can be considered as minimum estimates.

Due to the low level (<1%) of inter-atoll migration recorded, it appears unlikely that the higher population at Boduhithi is due to immigration of manta rays from outside the atoll during the NE Monsoon. Furthermore, almost 50% of manta rays sighted more than once in North Male atoll were recorded at both Boduhithi and Lankan sites, alternating between them in the NE and SW Monsoons, respectively.

The population estimates determined using Petersen's method (2) and using the Jolly-Seber model based on Lankan Reef, and combined data were significantly smaller than using Petersen's method (1) (one way ANOVA: F=7.71, p=0.011; F=6.77, p=0.004 respectively). The mean estimates using the Petersen's method (2) and Jolly-Seber model on combined North Male data are 536 (SD±244) and 538 (SD±422). The Petersen's (2) and Jolly-Seber estimates were based on samples taken in a 13 month period so the atoll population could be considered closed and these estimates appear realistic as an annual population for the atoll. It appears that the Petersen (1) method is over-estimating populations and this is likely to be due to the assumptions of closure being violated by the length of time over which the marked sample was taken. There are 709 manta rays currently recorded from North Male and the discovery curve (Fig. 8) suggests that the North Male atoll population is unlikely to exceed 800 animals. The known migration level of <1% of manta rays seen in more than one atoll over nine years is unlikely to cause population numbers to vary significantly between surveys conducted months, or even years apart.

Despite the large number of surveys, survey effort is low compared to the available time that manta rays may visit cleaning stations and is estimated at around 2.5% of the time when mantas rays might be expected at the sites (the hours of daylight during the relevant monsoon season). However, up to 93 different manta rays were positively identified visiting a cleaning station during an intensive survey day (it is likely that a few others were present but not identified) suggesting that almost 20% of the estimated North Male atoll population (~540) may visit a single cleaning station in a day. With more intensive surveying it is likely that individual manta rays would be re-sighted more frequently. Taking these factors into account, the estimates of manta ray population in North Male obtained using the combined sites data using Petersen's (2) or Jolly-Seber models seems realistic.

Ari atoll

The discovery curve of new manta rays for Ari atoll shows no clear sign of reaching an asymptote, with a recorded population of over 750 mantas (Fig. 8). Estimates of the Ari atoll population could only be obtained using Petersen method (1) because only two full-day intensive studies were undertaken and no animals were seen on both occasions, thus precluding both Petersen method (2) and Jolly-Seber estimates. The lack of re-sightings was surprising because the surveys were undertaken only 30 days apart at two sites (Madivaru and Table Thila) approximately 42 km apart, which is within the known length of a daily swim by a *M. alfredi* (Dewar et al. 2008). The mean population estimate from the two-full day surveys using the Petersen (1) method was 1468. No data were available on SW monsoon cleaning stations so the results are confined to observations over the 5 months of the NE monsoon. Seven

mantas (<1%) sighted in Ari atoll have also been recorded in North Male during the SW monsoon, suggesting a low level of inter-monsoon movement between atolls.

Baa atoll

There was a greater variation in the Baa atoll results than observed in the other atolls which was unexpected as the intensive surveys were made on almost consecutive days, so the population could be considered closed compared to the other atolls (196 of the 231 known manta rays from the atoll were first discovered during the 16-day sampling period). Based on the atoll area, the mean atoll population of 719 (SD±631) using Petersen's method (1) for Baa atoll, is similar pro rata, with the estimate of 1026 (SD±474) for North Male atoll, which is about 1.7 times the area of Baa. The significantly lower estimate of 301 (SD±183) using method (2) is due to small samples (~10) being relatively frequently obtained (in calculation, two samples of 10 may give a maximum population of 100). The Jolly-Seber population estimates were even lower than those obtained with Petersen's models (1) and (2) and overall the three sets of estimates were significantly different ($F=7.46$, $p=0.002$). The small proportion of matches between surveys made only days apart and the wide range in population estimates suggest rapid circulation and dispersion of manta rays around this atoll. The mean estimated population using Jolly-Seber of 204 (SD±163), may be representative of the number of animals in the vicinity of Hanifaru (east Baa atoll) around the time of the survey dates, whilst the mean result of 719 obtained using Petersen method (1) may represent an estimate of the population of animals in the vicinity of the whole of Baa atoll. This result using Petersen (1) method might be considered more reliable than those made with this method for the other atolls as the Baa study was over a relatively short period of time (<2 years) so the population might be considered closed. The upper estimate of 588 by the Jolly-Seber method suggests that the majority of animals may have been in the vicinity of Hanifaru on that day. The discovery curve of newly identified individuals for Baa atoll is still increasing linearly with no evidence of any asymptote, indicating that the population is likely to be in excess of the 231 individuals so far identified.

Implications for Maldives population estimate

The 1835 manta rays recorded from the central atolls of the Maldives is the largest population of individually identified *Manta alfredi* in the world so far. The estimated atoll populations of 537 for small atolls and 811 for large atolls seem realistic based on the identified populations. The extrapolated estimated Alfred manta ray population of the Maldives of 9,677 animals may be conservative but provides a starting point for managing this resource.

In Mozambique, Marshall et al. (2011) re-sighted 40.5% of 449 identified *M. alfredi* and estimated annual population sizes of 149 to 454 individuals and a super-population of 802. The study was conducted over four years and gaps of up to almost 3.5 years occurred between sightings. The relatively infrequent re-sightings and long gaps between sightings are a common feature of the two studies. In contrast, Deakos et al. (2011) resighted 73% of their identified population of 290 manta rays in Maui, Hawaii. They estimated the annual population using the program CAPTURE with Cormack-Seber-Jolly model as 77–230 animals. In these studies, the maximum annual population estimate was close to the total number of identified animals (454 vs. 445 and 230 vs. 290 respectively).

The re-sightings rate in this study of 24.7% was the lowest of the three study locations and was probably due to the large geographical area covered in this study (the three atolls have a total area of approximately 6126 km^2) and that study in Baa atoll had only commenced in 2007. The highest rate of re-sighting (34.5%) was in North Male atoll where greatest survey effort was expended. Individual heterogeneity may include behavioural differences, segregation by sex, age, size etc. and differing amounts of time spent in the survey area (Buckland 1990; Guttridge et al. 2009). There is likely to be variation in capture probability and transience due to temporary emigration to other feeding and cleaning station sites within the atoll so the animal in unavailable for sampling. Individual heterogeneity in capture probability may result in increased re-capture of some individuals which negatively biases population estimates and vice-versa. The primary underlying assumption of the Jolly-Seber model is that there is equal catchability of marked and unmarked individuals (Schwarz and Arnason 1996). It is likely that individual

heterogeneity and transience observed in *M. alfredi* violate this assumption, with marked animals being more likely to be re-sighted than an unmarked animal, ever sighted, and causing a negative bias on population. The two cleaning stations at Lankan Reef and Madivaru are very frequently visited by tourist scuba divers and it is likely that the manta rays which visit the cleaning stations have become habituated to the presence of divers but conversely, that other rays do not attend the cleaning station as they are deterred by the presence of divers. The effect may be cumulative, with fewer manta rays from a smaller population of bolder animals visiting year on year. This may be a cause of the difference in estimated atoll population between Lankan and Boduhithi (North Male atoll), and between Madivaru to Table thila (Ari atoll). Madivaru is the most valuable manta ray cleaning station in the Maldives in terms of dollars revenue (Anderson et al. 2010). It is notable that Madivaru has the highest proportion of female manta rays (~70%) of all the cleaning stations (Hanifaru is principally a feeding site) and this may be due to the number of visiting divers deterring all but the boldest manta rays from visiting the site. Whilst this is unlikely to have a detrimental effect on the animals as there are many other cleaning stations nearby which are less accessible to divers, there is an impact on income from tourism if well known sites cannot be depended on for good manta ray sightings. It is in the interests of both the manta rays and dive operators that tourism at the important sites is managed.

The estimates of manta ray population for atolls and cleaning/feeding sites may be used, broadly, as a baseline for future studies on the impact of tourism at the main Maldivian dive sites. It is important that future studies take into account the variation in population estimates using different sites (local populations should be measured and compared) and effects should be measured over relatively long periods of time i.e. over a number of years to take into account the natural fluctuations reported in other studies (Deakos et al. 2011; Marshall et al. 2011).

Despite the study of manta rays in Komodo Marine Park, Indonesia by Dewar et al (2008) indicating high site fidelity, this is not what we experienced during consecutive day long observations included in this study. This was particularly apparent on the study of the east Baa atoll sites and of Hanifaru in particular where the majority of Baa atoll manta rays were reported. The sites were visited on consecutive days yet there were a low number of individuals re-sighted during the study, even on consecutive days despite large numbers of manta rays being reported. This pattern is seen in the other atolls in the relatively high proportion of manta rays sighted once only. Instead, the low frequency of visit to the cleaning stations or feeding area sites observed in this study suggests that there are likely to be many other cleaning or feeding areas of which we are currently unaware (as the animals are likely to feed and be cleaned daily), and that individual *M. alfredi* are highly mobile in the Maldives making accurate population estimation difficult. Wide dispersion has conservation considerations in slowly reproducing (estimated annual fecundity $<<1$ from authors' own unpublished data and Marshall and Bennett (2010b)), fishery-targeted species. Fortunately there is no targeted manta ray fishery in the Maldives and the rays do not appear to travel much beyond an atoll but conservation and management of manta ray populations in other areas will be essential to prevent local over-exploitation of this species.

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Appendix B. An example of matching using the Access database

The full method for the use of the database is described in Chapter 3 and the identification method is described in Chapter 4. This appendix works through the process of matching a photographed manta. An unidentified manta is known to have the following characteristics from the photo below (Figure B.1) and from notes made during the dive: female, 3.5 m DW, four ovals in Area 2 (ringed in red), long cut tail, marks at distal end of gill-slits not obvious.

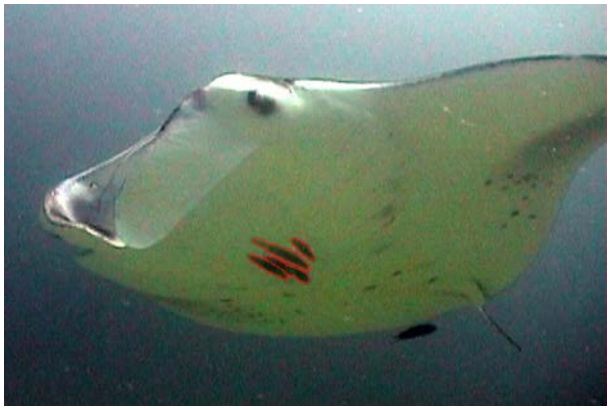

Figure B.1 Photograph of unknown manta to be identified. There are four ovals in Area 2 between the gills, ringed in red

These criteria can be entered into a query, asking a search of all females with those characteristics (Figure B.2).

Manta details Query 8 : Select Query

	Sex	Size	ID	Tail status	Area 1 spot #	Area 2 spot #
▶	Female	3.50	1	Long cut	0	4
	Female	3.50	447	Long cut	0	4
	Female	3.50	607	Long cut	0	4
	Female	3.50	631	Long cut	0	4
	Female	3.50	726	Long cut	0	4
	Female	3.50	1022	Long cut	0	4
	Female	3.50	1118	Long cut	0	4
*			toNumber)			

Figure B.2 Outcome of query for female 3.5m DW (size) long cut tail, zero spots in Area 1 and 4 spots in Area 2. The top line entry is used to define the search and is discounted. Six mantas are found on the database with these physical characteristics: IDs 447, 607, 631, 726, 1022 and 1118

The query produced just six known mantas (Figure B.2) with the physical characteristics of the unknown manta. The standardised drawings are now compared to the photograph in Figure B.1 to look for the best match (Figure B.3).

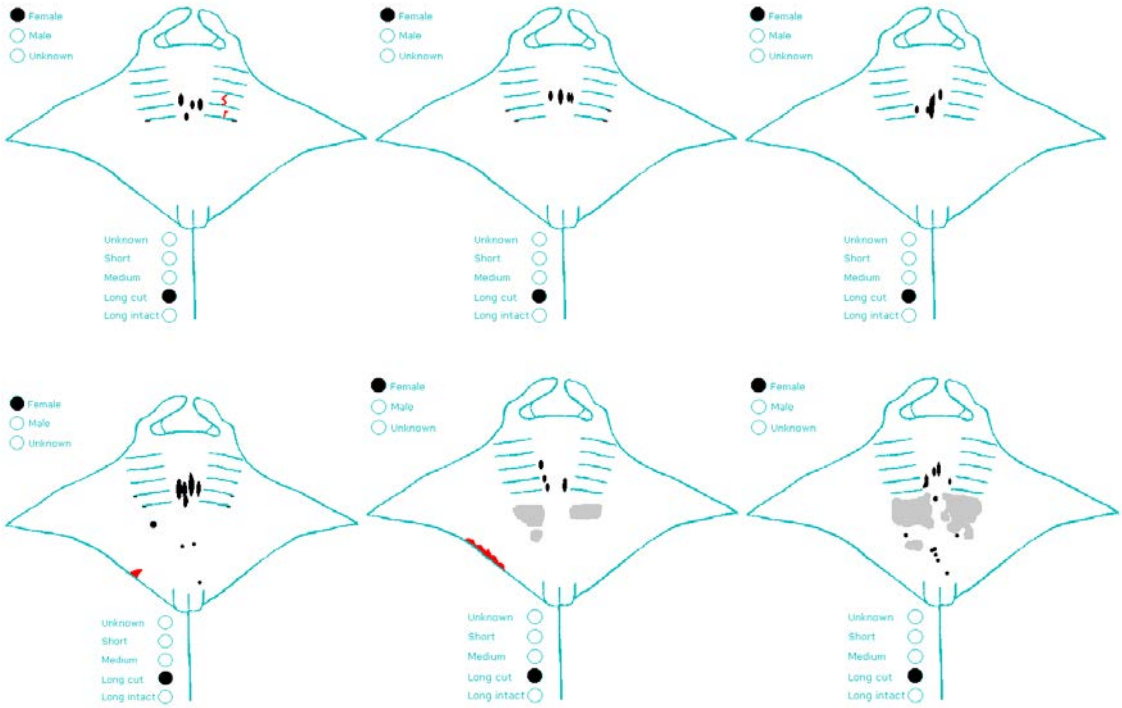

Figure B.3 Standardised drawings of (top line from left) 447, 607, 331 (bottom line from left) 726, 1022, 1118

The best match is obtained from the standardised drawing of 726 (Figure B.3, lower left) as the four ovals are long and clustered together with the oval to the right being slightly apart from the grouping of the other three. In addition the lower abdomen spots match the photograph so it is very likely there is a match. A final check is made using file photographs (Figure B.4). The marks between the gill-slits can be matched as can the spots on the abdomen so the unknown manta can be confirmed as manta 726 D78. The sighting is recorded in the comments box of 726 D78 and on a survey/encounter form.

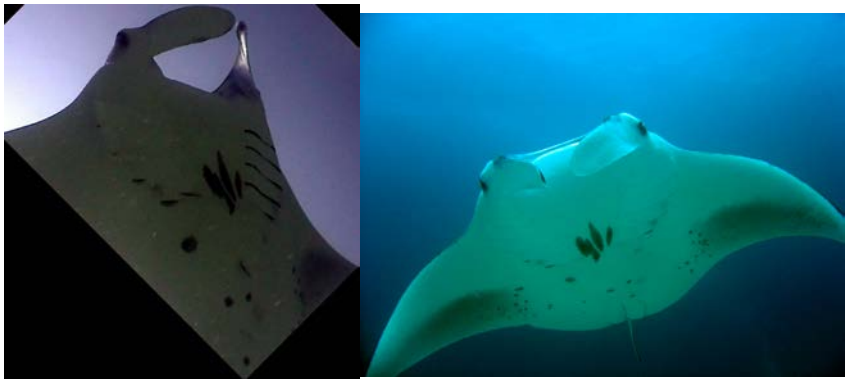

Figure B.4 File photographs of 726 D78 (full database name)

Appendix C. Mantas sighted at Lankan Reef during full day observations in 2006 and 2007

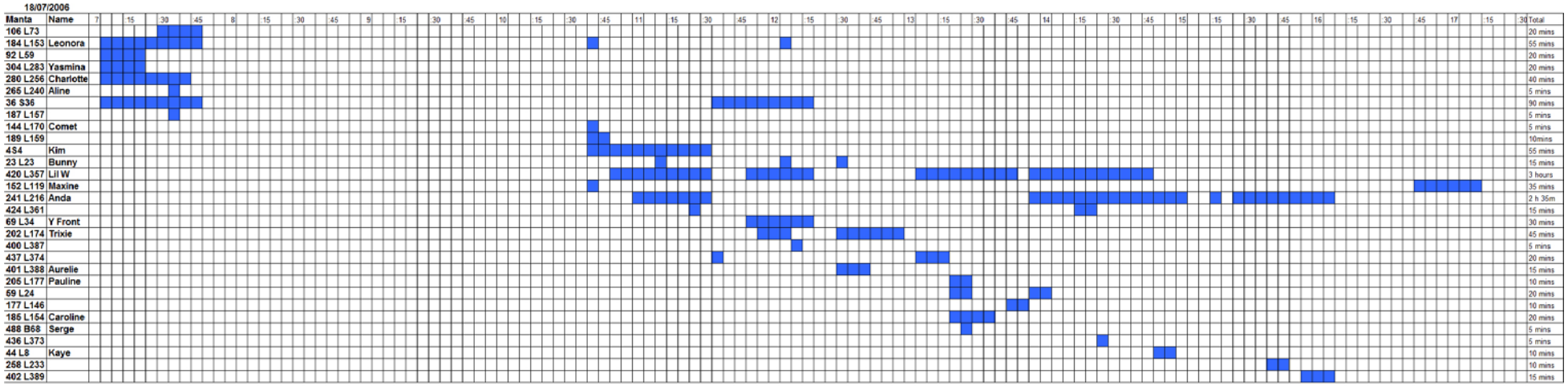

Figure C.1 Periods when mantas present at Lankan Reef cleaning station on 18/7/2006

Figure C.2 Periods when mantas present at Lankan Reef cleaning station on 26/7/2006

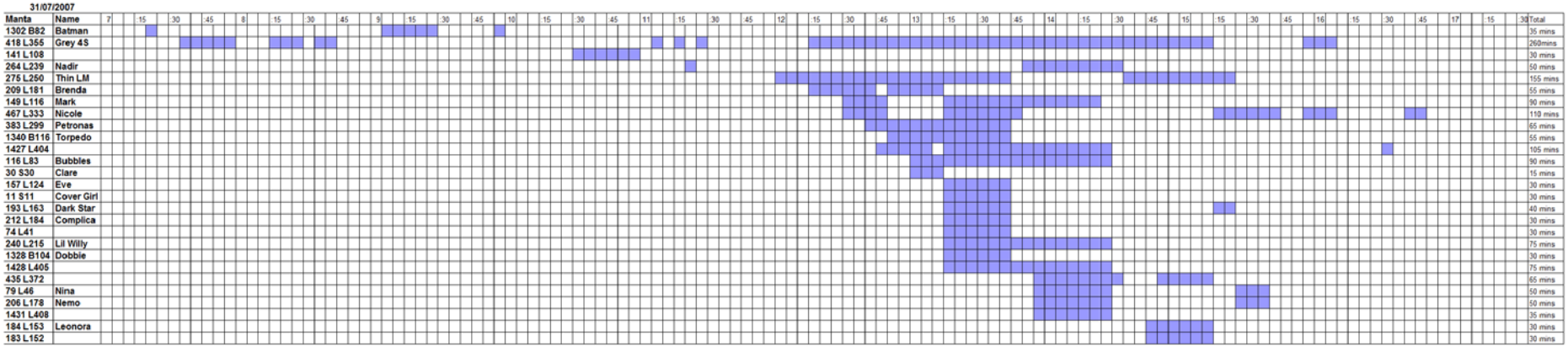

Figure C.3 Periods when mantas present at Lankan Reef cleaning station on 31/7/2007

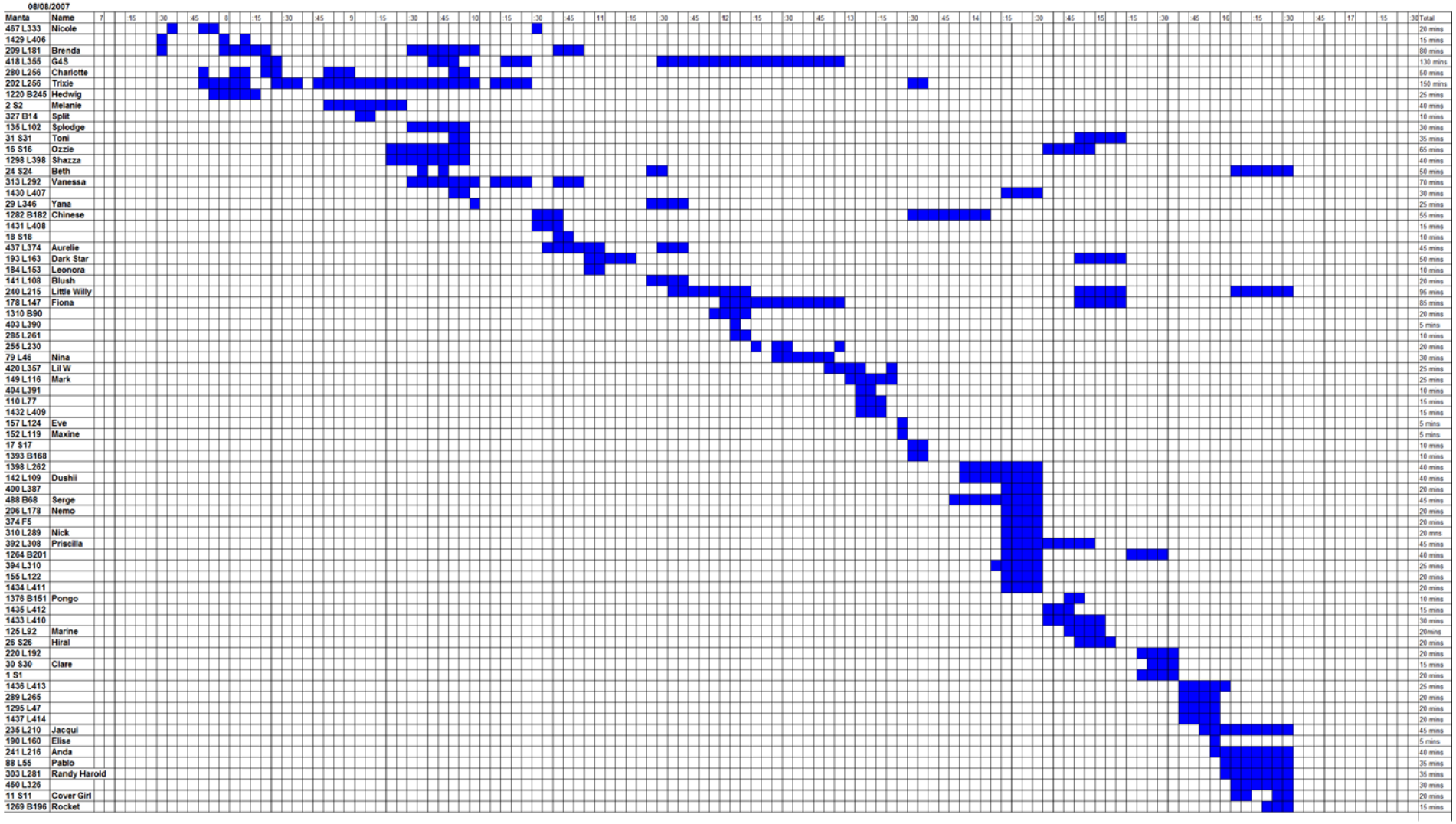

Figure C.4 Periods when mantas present at Lankan Reef cleaning station on 8/8/2007

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